perivascular space surrounding cortical blood vessels.

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A Comparative Study on the Virulence of *Mycoplasma arthritidis* And "*Mycoplasma hominis,* Type 2" Strains in Rats.* (31676)

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Mycoplasma hominis, type 2, and *M. arthritidis,* the causative agent of rat polyarthritis, have many common properties. Antigenic identity has been shown by complement fixation tests(1,2) although agar-gel double diffusion studies show minor differences between established laboratory strains(3). Pease reported that *M. hominis,* type 2, was antigenically identical with a strain of *M. arthritidis* which had recently been isolated by one of us from a bronchiectatic lesion in rat lung(4). On the basis of morphology and physiology, these strains are essentially the same(5,6). Finally, the pathogenic properties of *M. hominis,* type 2, for rodents were demonstrated by the production of a local abscess in mice following subcutaneous injection of the organism with agar(5). In 1965, Edward and Freundt proposed that *M. hominis,* type 2, be reclassified as *M. arthritidis*(6).

The above observations lend strong support to the identity of these strains. Nonetheless, additional evidence is desirable before final classification is accepted. We therefore have compared the pathogenicity of *M. hominis,* type 2, with a laboratory maintained and recent isolate of *M. arthritidis* for the rat.

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Materials and methods. Strains. M. hominis, type 2, strain 14152 (Edward strain PG 27, originally obtained from Dr. L. Dienes as strain 'Campo'), and *M. arthritidis,* strain 14124, were obtained from the American Type Culture Collection (Rockville, Md.). *M. hominis,* type 2, strain 158 (cloned 3 times from Dr. Edward's† strain PG 27) was obtained from Dr. M. F. Barile (Division of Biologic Standards, Bethesda, Md.). *M. arthritidis,* strain PN, was isolated by the authors from a subcutaneous nuchal abscess in a rat.

Physiology. PPLO (Difco Laboratories) agar and PPLO (Difco) broth, supplemented with 10% (v/v) horse serum (inactivated at 56°C for 30 minutes) were used throughout as the basal media. Carbohydrate fermentation was tested in broth containing 0.005% (w/v) phenol red and 1% (w/v) carbohydrate. Glucose, maltose, starch and dextrin were added from sterile 10% (w/v) Seitz-filtered solutions. Cultures were examined at intervals for 10 days of incubation for change of phenol red to a yellow color. Ammonia production was tested by adding one drop of Nessler's reagent to one drop of a 3-5-day broth culture containing 1% (w/v) arginine monohydrochloride. Lipolysis was determined by growth on agar containing

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10% (v/v) egg yolk suspension ('Oxoid,' Consolidated Laboratories, Inc.). The reduction of tetrazolium compounds was tested aerobically and anaerobically on agar containing 0.005% (w/v) triphenyltetrazolium chloride, and 0.00125% (w/v) tetrazolium blue(7).

Hemolysis was determined by growing the organisms for 48 hours on basal media containing 3% (v/v) guinea pig, sheep and rabbit bloods with both aerobic and anaerobic incubation.

Pathogenicity. Cultures were grown for 2 to 3 days in serum broth on a reciprocating shaker (Eberbach Corp., Ann Arbor, Mich.) at 37°C. The cells were harvested by centrifugation. The sedimented mycoplasma were resuspended in sterile serum broth to provide a concentration the same as the original culture (1×) or a 50-fold concentration (50×). Viable cell counts, expressed as colony forming units (CFU), were performed on these suspensions by the method of Miles and Misra just prior to injection(8). Albino rats (Holtzman) weighing 100-120 g were injected in groups of 10, intravenously (I.V.) or intraperitoneally (I.P.), with 0.5 ml amounts of the suspensions. The 50× suspensions were also injected subcutaneously into the shaved backs of each of 10 rats. Suspensions were also prepared for I.P. injection using 5% gastric mucin (Nutritional Biochemicals Corp., Cleveland, Ohio) which was sterilized by autoclaving. The animals were examined periodically for the development of arthritis or abscesses, for up to 14 days following injection. Upon completion of the experiments, the animals were sacrificed and representative joints, abscesses, and organs were cultured on basal media for mycoplasma. The severity of arthritis was recorded by scoring each infected joint from 1-4(9). The mean score of infected animals for each group was determined at the time of maximum arthritis for each animal.

Results. Physiology. All strains grew well on serum agar and were characterized by smooth colonies with well-defined central regions of growth into the agar. Carbohydrates were not attacked and no lipolytic activity was evident on horse serum or egg yolk agar.

TABLE I. Virulence of Mycoplasma for Rats.

Mycoplasma strain and route of inj	Sex	Inoculum/animal (CFU)*	Arthritis	Paralysis	Mean score of infected rats	Mean No. of extremities involved/rat	Abscess on S.C. inj	Recovery of mycoplasma from joints
M. arthritidis, ATCC 14124, I.V.	♂	50× (2 × 10^9)	3/10	0/10	4	1	8/10	—
Idem	♀	50× (1.5 × 10^{10})	10/10	1/10	26	3	9/10	3/3
M. arthritidis, ATCC 14124, I.P.	"	50× (1.5 × 10^{10})	8/10	1/10	12	2	—	—
M. arthritidis, ATCC 14124, I.P. + mucin	"	50× (1.5 × 10^{10})	7/10	2/10	30	3	—	—
M. hominis 2, ATCC 14152, I.V.	♂	50× (1 × 10^{10})	4/10	1/10	4	1	10/10	—
M. hominis 2, ATCC 14152, I.P.	♀	50× (4 × 10^{10})	5/10	0/10	23	4	—	2/3
M. hominis 2, ATCC 14152, I.P. + mucin	"	50× (4 × 10^{10})	6/10	2/10	12	2	—	—
M. hominis 2, 158, I.V.	♂	50× (1.7 × 10^{10})	8/10	1/10	12	3	9/10	—
PN, I.V.	♀	1× (4 × 10^7)	9/10	3/10	18	2	—	—
Idem	"	50× (2 × 10^9)	9/10	3/10	22	3	10/10	3/3

* CFU = colony forming units (see text).

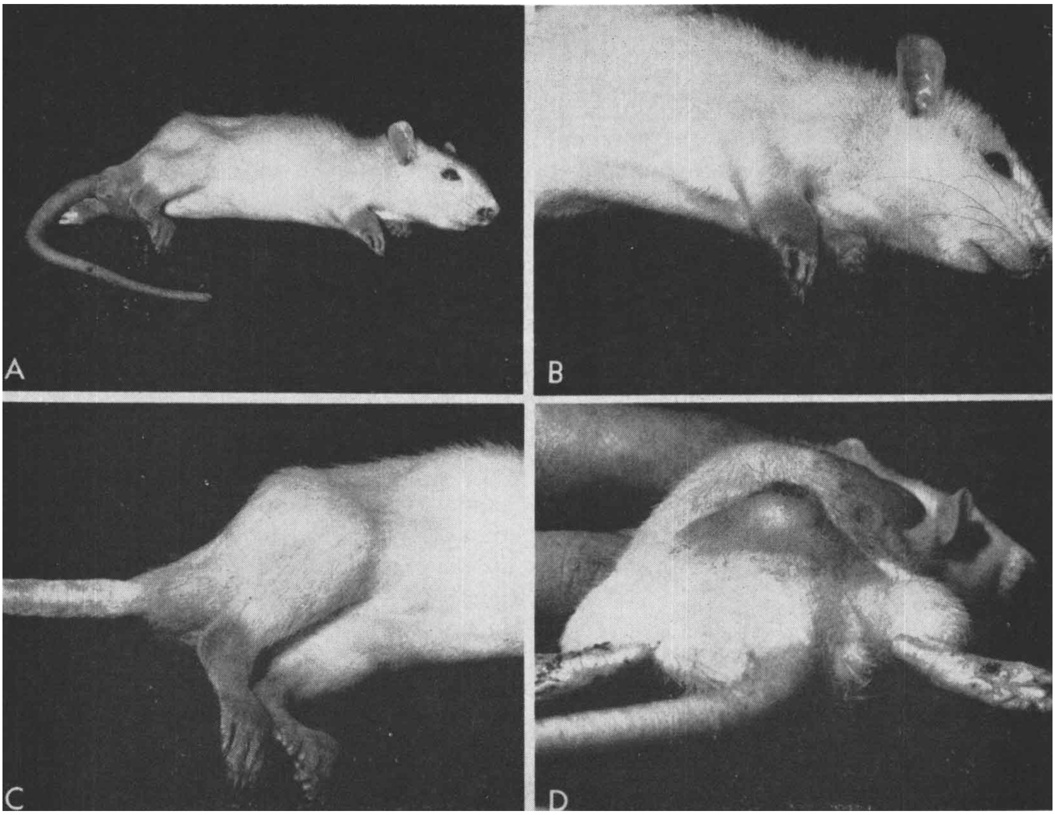

FIG. 1. Photographs of rats injected with mycoplasma. A. Arthritis involving all 4 extremities in animal injected with 2 x 10^9 CFU *M. arthritidis*, strain PN. B & C. Close-up views of forepaws and hindpaws showing extensive arthritis. D. Abscess produced by S.C. injection of 1.7 x 10^{10} CFU *M. hominis,* type 2, strain 158.

All strains produced ammonia from arginine and reduced triphenyltetrazolium chloride and tetrazolium blue anaerobically, but not aerobically. No hemolysis was observed with sheep and rabbit erythrocytes. *M. arthritidis,* strain 14124 and strain PN, produced β-hemolysis of guinea pig erythrocytes on aerobic incubation; *M. hominis,* type 2, strain 14152, showed weak β-hemolysis of guinea pig erythrocytes anaerobically, but not aerobically.

Pathogenicity. The results are summarized in Table I. Inocula of 1$\times$ suspensions of *M. hominis,* type 2, strain 14152 and 158, and *M. arthritidis,* strain 14124, did not produce arthritis upon I.V. or I.P. injection. Similarly, no arthritis developed with direct broth cultures of these strains. On the other hand, there was a high incidence of arthritis when similar numbers of cells of the recent isolate, strain PN, were injected.

The 50-fold concentrated suspensions of *M. hominis,* type 2, and *M. arthritidis* produced arthritis on I.V. and I.P. injection, and 80-100% of animals developed abscesses on subcutaneous injection. On the basis of the results obtained, the approximate ID_{50} of these organisms would lie between 2 $\times$ 10^9 and 4 $\times$ 10^{10} CFU. For strain PN, however, the ID_{50} would be below 4 $\times$ 10^7 CFU.

Mucin, which has been used extensively to enhance the virulence of bacteria for laboratory animals, did not produce a significant increase in the incidence of arthritis with either the 1$\times$ or the 50$\times$ suspensions(10).

Mycoplasma could readily be isolated on serum agar from arthritic joints, and subsequent physiological tests on these isolates showed them to be identical with the strains injected. Isolations from blood, lungs, kidney, spleen, liver and peritoneal lymph node were attempted from arthritic animals injected I.P. with *M. hominis,* type 2, strain 14152. A few colonies morphologically resembling the injected strain were detected in the lung and liver cultures. None were present in similar isolations from 3 normal animals. Colonies resembling *M. pulmonis* were occasionally encountered in the lungs, kidney, spleen and liver cultures of both groups. Autopsies showed that rats injected I.P. developed small abscesses on the parietal peritoneum at the site of injection; mycoplasma could readily be isolated from these abscesses. All colonies of suspected mycoplasma were examined on agar plates with a stereoscopic microscope at 30× magnification, and isolated colonies were stained and examined by the method of Dienes(11). Bacteria were never isolated from arthritic joints.

The arthritis which developed closely resembled the arthritis produced by a virulent fresh isolate (Fig. 1)(12). Arthritis usually appeared 3 to 4 days following injection, but with laboratory-maintained strains of mycoplasma and lower doses of inocula, arthritis occasionally did not appear until the 7th or 8th day. The arthritis usually started to remit between the 10th and 14th day following injection. Conjunctivitis, opacity of the eye, and nasal discharge were sometimes present in severe cases. Paralysis, usually of the hind limbs, often developed in severe cases, but sometimes occurred in animals which showed no symptoms of arthritis. Paralysis was noticeably higher with the resent isolate, strain PN, than with the other strains. A yellow, or yellow-green, pus was invariably present in severely infected joints, and in some cases, abscesses were present on the joints.

The abscesses which developed on subcutaneous injection reached a maximum size of approximately 3.0 cm in diameter after the 7th to 14th day following injection. They were characterized by a yellow-green pus from which mycoplasma could readily be isolated.

Discussion. In a careful review, Edward and Freundt have discussed the status of *M. hominis,* type 2, and its relationship to *M. arthritidis*(6). The physiologic similarities between the *M. hominis,* type 2, strains and *M. arthritidis* strains observed in this study confirm the findings of other workers. The differences between the strains in the hemolysis tests was of interest, but this could represent strain variation and is not a basis for species differentiation.

The production of arthritis by the two strains of *M. hominis,* type 2, studied further supports the identity of these organisms with *M. arthritidis.* In this context, it is interesting to note that the *M. hominis,* type 2, strains which have both been maintained for long periods in various laboratories possessed the same degree of virulence as *M. arthritidis,* strain 14124, which is also a laboratory-maintained strain. Loss of virulence on artificial passage has been described for *M. pneumoniae,* and would account for other workers' difficulties in inducing rat arthritis by using direct broth cultures of *M. arthritidis*(13,14).

The ability of the *M. hominis,* type 2, strains to produce abscesses upon subcutaneous injection further illustrates the similarity in pathogenicity of these organisms for rats. Whereas some workers have found an agar adjuvant necessary to induce abscesses with *M. arthritidis*(15), this was not found to be required in the present experiments.

Summary. The arthritogenic properties of 2 strains of *M. hominis,* type 2, were demonstrated. The virulence of these was comparable to that of *M. arthritidis,* strain ATCC 14124, but was less than that of a recent rat isolate, strain PN. The results support the identity of *M. hominis,* type 2, with the rat species, *M. arthritidis.*

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Cloning of Immunoglobulin-Producing Human Leukemic and Lymphoma Cells in Long-Term Cultures.* (31677)

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Immunoglobulin (Ig) production in a large number of *in vitro* cultured human leukemia and Burkitt lymphoma cell lines has recently been reported(1,2,3,4). These studies indicated that the cell lines produced diverse immunoglobulins. Immunofluorescent studies of smears of these cell lines demonstrated that the specific immunoglobulin staining occurred in the cytoplasm of many cells, although there were always some cells which showed no staining. These observations suggested a possible heterogeneity of these cell lines with respect to immunoglobulin production and our cloning studies were undertaken in an effort to elucidate this question.

Very recently a technique for deriving single cell clones from a line of Burkitt lymphoma cells by a semi-solid agar procedure was developed in this laboratory(5). The present communication describes successful cloning of 2 lines of human leukemia cells and 2 lines of Burkitt lymphoma cells and an immunofluorescent analysis of the clonally derived cell strains with respect to their production of heavy chain immunoglobulins.

Materials and methods. The cell lines used in these studies were the 64-10 line derived from a patient with myelogenous leukemia by Iwakata and Grace(6); the LKID line derived from a patient with lymphocytic leukemia by Armstrong(7); two lines designated SL-1 and P3J isolated from African patients with Burkitt lymphoma by Stewart *et al*(8) and Pulvertaft‡ respectively. All lines were carried in this laboratory as stationary suspension cultures in a medium composed of 80% Eagle's minimum essential medium and 20% fetal calf serum plus 20 μg/ml of L-serine and 110 μg/ml of sodium pyruvate. The cell cloning procedure is described in more detail elsewhere(5). The above growth medium was used for the cloning but with the addition of 0.4% and 0.3% of ethanol-ether washed Bacto-agar for the base and the seed layers respectively. Five ml of the base agar was solidified in a plastic petri dish (60 $\times$ 15 mm). Two or three days after subculture of the cells in growth medium in a 5-7% CO_2 humidified incubator the cells were dispersed singly by pipetting and diluted with the seed agar to concentrations of 100 cells and 10 cells per ml respectively. Two ml of each dilution were layered onto the base agar in a petri dish. The inoculated dishes were then incubated in the CO_2 incubator.

Within 7 to 10 days after plating, colony formation was observed with all 4 cell lines. The number of colonies was counted under a dissecting microscope. The cloning efficiency of each cell line was 6% for 64-10, 13% for LKID, 21% for SL-1 and 72% for P3J. The cloning efficiency of each cell line was independent of the initial cell density and a dilution effect on the number of colonies formed was observed providing evidence that

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