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Factors Influencing Pathogenicity of *Mycoplasma arthritidis* (PPLO).* (27952)

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Mycoplasma, (pleuropneumonia-like organisms, PPLO) pathogenic in many animal species, were first reported to be a causal agent of rat polyarthritis in 1939(1). In a previous study(2), rats bearing a lymphosarcoma developed sporadic polyarthritis during or after regression of the tumor. When PPLO were recovered from tumor or joint lesions, and injected into PPLO-free rats, polyarthritis was produced in those rats with necrotic or recently regressed tumors, but not in normal rats or in those with intact tumors. Additional studies suggested that both host and agent factors were significant in the pathogenesis of the PPLO polyarthritis(3). As one phase in the investigation of the pathogenesis of mycoplasmal arthritis, the present study concerns the modification of virulence of Mycoplasma *in vitro* as well as *in vivo*.

Materials and methods. PPLO, recovered from a joint lesion of a rat with a regressed Murphy-Sturm lymphosarcoma, were maintained by infecting the transplantable lymphosarcoma in young male Holtzman rats, 120 to 170 g in weight, as previously described (2). PPLO from tumor tissue were isolated on blood agar plates. Small segments of agar with large numbers of PPLO colonies were inoculated into Difco PPLO broth. Broth subcultures were made at weekly intervals using a 20% inoculum from the previous subculture and incubating for 5 to 7 days at 37°C. Aliquots were injected intravenously into rats, 0.5 ml/100 g body weight. The criterion for mycoplasmal pathogenicity was the production of gross joint lesions.

Tumor extract was prepared by aseptically removing growing lymphosarcoma, mincing in an equal volume of balanced salt solution (Earle's), and grinding in a Waring blendor. Tissue fragments, cells and extraneous bacteria were removed by Seitz filtration. The tumor extract was added as a supplement in 10% concentration to Difco PPLO broth

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base. Non-pathogenic PPLO obtained by repeated subculture, and pathogenic PPLO grown in the supplemented media, were tested for arthritogenic properties by intravenous injection into normal rats. To exclude a possible role of tumor extract in the mycoplasmal polyarthritis, tumor extract was added to a portion of the 6th subculture of PPLO in Difco broth immediately before being injected intravenously into a group of rats.

Modification of virulence of PPLO *in vivo* was evaluated by intravenous injection of PPLO, non-pathogenic in normal rats, into rats bearing an intact PPLO-free lymphosarcoma 25 mm in mean diameter. Partial necrosis of these tumors was then induced by 4 daily intravenous injections of Klebsiella polysaccharide, 0.1 mg/100 g, as described previously(4). PPLO were reisolated from the tumor tissue, cultured in Difco broth and injected into a group of normal untreated rats. Klebsiella polysaccharide was also injected into normal, tumor-free rats prior to intravenous introduction of PPLO.

Two morphological types of Mycoplasma, designated "clonal" and "diffuse," were isolated from the original infected tumors. Each of these morphological types were subcultured in Difco broth supplemented with tumor extract. These "clonal" and "diffuse" types were injected into separate groups of rats and the incidence and distribution of arthritic lesions observed.

Serum was obtained from normal rats and from rats which had recovered from severe arthritis and were resistant to subsequent injections of virulent PPLO. From such animals splenic cells were also obtained aseptically, suspended in Earle's solution, counted in a hemocytometer, diluted to desired concentration and immediately injected intravenously, 5.0×10^6 cells per 0.1 ml per 100 g body weight. Following intravenous injections of the 2 sera, 0.5 ml/100 g body weight, and the 2 cell suspensions into separate groups of normal rats, pathogenic PPLO were injected by the same route 1 and 7 days later.

Results. By repeated subculture in Difco broth, virulent PPLO became non-pathogenic, but pathogenicity was both maintained and restored by subculturing in media supplemented with the cell-free tumor extract (Table I). Tumor extract added to non-pathogenic PPLO immediately before injection did not increase the incidence of arthritis.

TABLE I. Effect of Subculture of PPLO in Tumor Extract Broth and in Difco PPLO Broth Upon Virulence of PPLO in Normal Rats.

Subculture	Difco PPLO broth	Tumor extract broth
1	19/20*	20/20
3	1/20	17/20
6	0/20	8/10
6th Difco broth subculture + tumor extract	0/20† (not incubated)	7/20 (incubated)

* No. rats with joint lesions/No. rats per group.
† Tumor supplement added to 6th PPLO subculture (Difco broth) and without incubation immediately injected intravenously.

Mycoplasma, injected after repeated subculture in Difco broth without tumor extract, produced no polyarthritis in normal rats; however, arthritis was produced by these Mycoplasma in rats with recently regressed lymphosarcomas (Table II). The Klebsiella polysaccharide used to induce the tumor regression did not modify the host *per se,* for in tumor-free rats repeated injection of Klebsiella polysaccharide did not enhance the arthritogenic properties of these Mycoplasma (Table II). Pathogenicity was restored to the Mycoplasma by *in vivo* growth within the tumor. When the non-pathogenic Mycoplasma were injected intravenously into rats with focally necrotic lymphosarcoma, no arthritis developed. However, when these Mycoplasma were recovered from the tumor tissue by isolating on agar plates and subcul-

TABLE II. Effect of Subculture of PPLO in Difco Broth Upon Virulence of PPLO in Normal and Tumor-Bearing Rats.

	Normal		Tumor-bearing	
Subculture	Untreated	Polysaccharide-treated	Intact tumor	Regressed tumor
3rd	0/14*	0/14	0/14	12/14
6th	0/20	0/10	0/20	0/20

* No. rats with joint lesions/No. rats per group.

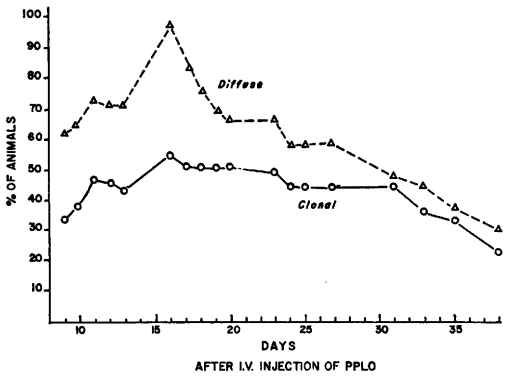

FIG. 1. Incidence of interphalangeal arthritis following injection of "diffuse" (△--△) or "clonal" (○—○) forms of PPLO.

turing in Difco PPLO broth without tumor extract, polyarthritis followed their injection into normal rats.

Virulent PPLO failed to produce arthritis in the 2 groups of rats which had recovered from mycoplasmal arthritis (Table III). Arthritis was prevented by passive transfer into normal rats of plasma from immune, but not from control animals (Table IV). Transfer of splenic cells of immune or control donors, either 1 or 7 days before injection of virulent Mycoplasma, did not prevent the arthritis.

When the 2 morphological types of Mycoplasma were injected respectively into 2 groups of rats, the diffuse form produced about twice the amount of interphalangeal polyarthritis (Fig. 1).

Comments. The data of the present study suggest that the pathogenesis of rat polyarthritis due to *Mycoplasma arthritidis* was influenced by the agent as well as the host. Although loss of virulence of certain bacteria after subculturing is a well-known phenomenon, the mechanism of such change remains obscure. The pathogenic Mycoplasma obtained from Murphy-Sturm lymphosarcoma, despite abundant growth upon subculture in commercially available media, lost the capacity to produce arthritis in rats. That an unknown nutritional requirement was needed for pathogenic propensity was suggested by restoration and maintenance of pathogenicity with a cell-free, sterile extract of tumor tissue. The nature of the component(s) in the extract is unknown but may represent the same "influences" noted in the reestablishment of pathogenicity of PPLO within the partially necrotic tumor *in vivo*. Since the necrotic and regressed tumor often increased the host susceptibility to Mycoplasmal arthritis, the tumor extract *in vitro* could, upon injection, have an action similar to that of the necrotic tumor *in vivo*. This possibility was excluded by the addition of tumor extract immediately before injection of the nonpathogenic PPLO in Difco broth (Table I). Joint lesions did not develop unless the PPLO were incubated in the combined media. These observations would also tend to exclude the possibility of arthritis being due to a viral agent transmitted with tumor or tumor extract.

The presence of intact, growing tumor tended to prevent the development of arthritis(5), while regression of a PPLO infected tumor was followed by polyarthritis(2). Furthermore, regression of PPLO-free tumor predisposed to arthritis following intravenous injection of mildly virulent PPLO(2). Pathogenic PPLO maintained in one of 2 transplantable lymphosarcomas in the same rat would readily pass from PPLO-infected to PPLO-free tumor without the advent of

TABLE III. Resistance to Reinfection with Virulent PPLO After Previous Mycoplasmal Polyarthritis.

Previous polyarthritis (healed)	Arthritic lesions
Spontaneous recovery	0/7*
Tetracycline-treated	0/7
None (control)	8/10

* No. rats with joint lesions/No. rats per group.

TABLE IV. Passive Transfer of Resistance to Virulent Mycoplasma.

Transferred material	Condition of donor			
	Normal		PPLO-immune	
	1 day*	7 days	1 day	7 days
Splenic cells	6/10†	8/10	7/10	7/10
Serum	7/10	6/10	0/10	0/10

* Day cells or serum transferred before injection of virulent PPLO.

† Control given PPLO only, 7/10.

arthritis(3). Despite the apparent lack of immunity to PPLO growing in tumor tissue, rats bearing PPLO-infected or PPLO-free tumors were relatively resistant to PPLO polyarthritis. Tumor transplants to rats with established chronic PPLO polyarthritis, did not become infected with PPLO(3). The present study suggests that circulating antibodies prevented spread of PPLO to the recently implanted tumor.

The role of the 2 morphological types of PPLO in polyarthritis is uncertain. No attempt was made to establish their interconversion and both types were pathogenic although the diffuse form produced more lesions. Following intravenous injection, pathogenic Mycoplasma rapidly disappeared from tissue but apparently lodged in synovial structures, establishing suppurative arthritis (6). Because of the unreliability of cultural and turbidometric methods for estimating numbers of PPLO the role of aggregation of these minute microrganisms upon their tissue distribution after intravenous injection, and hence upon the incidence of joint disease, remains unknown.

Summary. The ability of Mycoplasma (PPLO) to produce polyarthritis in rats has been modified *in vitro* and *in vivo*. Pathogenic PPLO recovered from Murphy-Sturm lymphosarcomas became non-virulent upon repeated subculture in commercial media, but the pathogenic properties were restored and maintained by addition of cell- and bacteria-free extract of the tumor tissue. Growth of Mycoplasma in tumor tissue *in vivo* also restored the pathogenic property. Diffuse and clonal forms of the pathogenic PPLO were isolated. Twice as much interphalangeal polyarthritis followed injection of the diffuse as the clonal form. Prior PPLO arthritis resulted in immunity. Passive transfer of the immunity was accomplished with serum but not with splenic cells.

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Electrophoretic Studies of Red Cell Hemolysates Supplemented with Phosphorylated Carbohydrate Intermediates.* (27953)

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During the storage of blood at 4°C in acid citrate dextrose (ACD) preservative, concentrations of the organic phosphates, particularly 2,3-diphosphoglycerate and adenosine triphosphate, of the red cell decrease and inorganic phosphate increases(1). Under these same conditions of storage, one of the electrophoretic components of the red cell hemolysate, designated as B, gradually disappears(2). It is conceivable that this component may be composed of a complex made up of phosphorylated carbohydrate intermediates and hemolysate proteins. The present experiments were undertaken to determine if the distribution of the components of the electrophoretic patterns of red cell hemolysates is changed after adding phosphoric acid esters.

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Materials and methods. The red cells of ACD blood, stored at 4°C for about a month, were washed 3 times with cold 0.9% NaCl and then hemolyzed with distilled water. Stroma was removed by high speed