Mycoplasmal (PPLO) Polyarthritis and Tumor Regression in Rats.*† (25195)

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Mycoplasma arthritidis, a pleuropneumonia-like organism (PPLO), has been reported to be a causal agent of polyarthritis in rats. Woglom and Warren encountered a filterable agent from abscesses occurring at the site where rat sarcoma had been transplanted(1). When this material was injected intravenously into normal rats, most of the surviving animals developed polyarthritis. Klieneberger (2) and Woglom and Warren(3) subsequently demonstrated the filterable agent to be a PPLO. Findlay, *et al.*(4) reported isolation of PPLO from affected joints of rats with spontaneous polyarthritis. Arthritis was produced by injecting sterile agar and a filtrate of the ground infected joints or a rabbit blood culture of PPLO into the pad of the hind foot of normal rats. Similarly, polyarthritis in normal rats has been produced by intracardiac injection of large volumes of an ascitic fluid broth culture of PPLO of the L4 type(5) or by intraperitoneal injection of broth cultures of PPLO(6). Jasmin(7) produced a proliferative arthritis in rats by intraperitoneal or intravenous injection of exudate collected from an air pouch containing lymphosarcoma. When this exudate was cultured in chick embryos a pleomorphic organism was isolated which, when subcultured on solid media, was similar to the L4 strain of PPLO. The sporadic occurrence of polyarthritis in rats during regressing of Murphy-Sturm lymphosarcoma induced by *Klebsiella pneumoniae* polysaccharide has been observed in our laboratory. Cultural studies yielded growth of PPLO, *Mycoplasma arthritidis*, from the involved periarticular tissues and the necrotic tumor. The present study was undertaken to investigate the respective roles of the PPLO, the tumor necrotizing bacterial polysaccharide, and the tumor regression in production of the polyarthritic lesions.

Material and methods. The Murphy-Sturm lymphosarcoma was propagated in male Sprague-Dawley rats weighing 90 to 110 g. Tumor tissue was aseptically minced, suspended in Earle's solution, and injected subcutaneously into the lumbo-spinal region. A palpable tumor appeared at site of injection in 6 to 10 days and grew rapidly to a spheroid mass of about 60 g measuring approximately 50 × 70 mm before death of the rat. Occasionally the tumor regressed spontaneously before reaching a diameter of 30 × 35 mm. Necrosis of the tumors was produced by 4 daily intraveneous injections of *Klebsiella pneumoniae* polysaccharide, 0.1 mg/100 g body weight. If the tumors were less than 35 mm in diameter, disappearance of the tumors usually resulted. Preparation of the polysaccharide has been described(8). PPLO were isolated and transplanted on modified Shepard's media(9) using Fields' tryptic digest broth (B-B-L). Colonies were examined by the stained cover-slip agar block method of Dienes(10). Difco PPLO broth, supplemented with phenol red dextrose broth and PPLO serum fraction, was used for fermentation studies. "PPLO-free" tumor-bearing rats were obtained by subcutaneous injections of 3.3 mg of tetracycline HCl twice daily for 7 to 10 days, beginning on the day of or the day before tumor transplant. These rats showed no arthritic lesions and their tumors were culturally negative for PPLO. On the 8th or 9th day following tumor transplant, 0.5 ml of a 4 day broth culture of PPLO was injected by tail vein. The first injection of polysaccharide was on the 10th or 11th day after tumor transplant. To evaluate the interrelationship of the 3 variables—PPLO, bacterial polysaccharide, and tumor regression—9 groups of male rats of similar age were used (Table I). Groups 1 through 4 were tumor-bearing rats. Of these, groups 1 and 2 were "PPLO-free," and 3 and 4 were PPLO-

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TABLE I. Rat Tumor Regression and PPLO-Arthritis.

	Tumor bearing rats				Non tumor controls		Prior (spontaneous) tumor regression
	PPLO-free		PPLO-inj.		PPLO-free	PPLO-inj.	PPLO-inj.
	Tumor regression						
	Pos.	Neg.	Pos.	Neg.			
	(Group 1)		(Group 3)		(Group 5)	(Group 7)	
Polysaccharide	*0/9	0/3	6/7	6/9	0/10	0/16	
	(Group 2)		(Group 4)		(Group 6)	(Group 8)	(Group 9)
No polysaccharide	0/1	0/9	4/4	4/11	0/10	0/10	9/9

* Numerator = No. of rats with arthritis. Denominator = No. of rats in group.

injected. Groups 1 and 3 received polysaccharide. Groups 5 through 8 were non-tumor control animals treated as in Groups 1 through 4. In Group 7, PPLO were injected into 10 rats 3 days prior to polysaccharide injection, into 3 rats on the third polysaccharide injection day, and into 3 additional rats on the day following the fourth polysaccharide injection. In addition, 9 rats (Group 9) with spontaneously regressed tumors and "PPLO-free" were injected with PPLO on the 5th to 8th day after onset of tumor regression.

Results. Twelve "PPLO-free" tumor bearing rats were injected with polysaccharide (Table I, Group 1). The tumors of 9 regressed; the other 3 showed only temporary inhibition of tumor growth. No arthritis was observed in any of the 12 animals. Similarly, arthritis did not develop in the 10 "PPLO-free" tumor-bearing rats which received no polysaccharide. Spontaneous tumor regression occurred in one of these animals (Table I, Group 2).

In the PPLO-injected animals, Groups 3 and 4, tumor regression was likewise more frequent following injections of polysaccharide. In Group 3, tumor regression occurred in 7 of 16 rats. In Group 4, the tumors of 4 of the 15 rats regressed spontaneously. In Groups 3 and 4, arthritis was seen in all the rats with tumor regression except one. In Group 3, nine rats had only temporary inhibition of tumor growth (negative tumor regression); 6 had arthritic lesions with extensive areas of necrosis in the tumor.

In the non-tumor control groups, no arthritis was observed in the PPLO-free or PPLO-injected groups with or without polysaccharide (Table I, Groups 5, 6, 7, 8). In contrast, 9 "PPLO-free" tumor-bearing rats with spontaneous tumor regression developed polyarthritis within 2 to 4 days following injection of PPLO (Table I, Group 9).

PPLO were recovered from all arthritic joints cultured; likewise, cultures from tumors of PPLO-injected rats yielded PPLO growth.

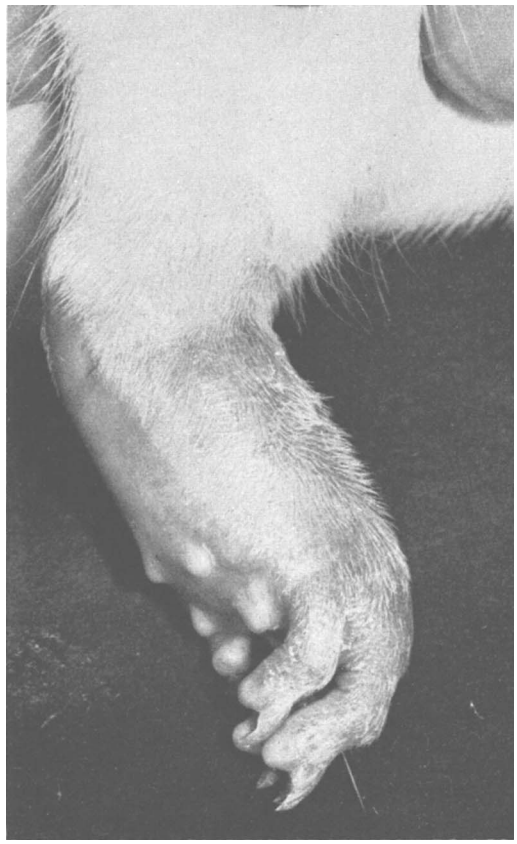

FIG. 1. Periarthritis in hind foot of rat with prior regressed lymphosarcoma. Arthritis developed 4 days after intravenous injection of *Mycoplasma arthritidis* (PPLO).

No PPLO were recovered from joints or tumors of "PPLO-free" tumor-bearing rats or from joints or tissue of non-tumor control rats.

Comments. Our data indicate that the significant condition for production of PPLO arthritis in rats was the concurrent or prior necrosis of Murphy-Sturm lymphosarcoma. Since polyarthritic lesions resulted from PPLO infection of tumor regressed rats, neither the bacterial polysaccharide, viable tumor nor actual necrotic tumor appears essential for induction of arthritis.

Production of acute joint lesions by bacterial polysaccharide in normal guinea pigs (11) suggested a similar role in the arthritis of tumor-bearing rats. However, in PPLO-injected rats without tumors, injection of bacterial polysaccharide resulted in no arthritis. That PPLO might localize in the articular structures only during a transient favorable phase following polysaccharide injection seems untenable since the polysaccharide was introduced at various intervals before and after PPLO injection. Likewise PPLO-infected arthritic lesions were observed in tumor-regressed rats that received no polysaccharide.

Although the arthritis occurred occasionally in some rats with progressively enlarging lymphosarcoma, areas of necrosis were found within such tumors. It should be emphasized that arthritis was much more frequent in the rats with regressing than with progressing tumors. Since in the PPLO infected animals the best source of the microorganisms was from tumor tissue, it may be proposed that the necrosis of the tumor leads to the massive release of PPLO into the blood stream with subsequent localization in articular or periarticular tissues. However, release of PPLO from necrotic tumor tissue would not appear essential inasmuch as the non-infected rats with complete, though recent, spontaneous regression of tumor developed arthritis within 2 or 3 days after intravenous injection of PPLO.

Various studies suggest that PPLO arthritis in rats may occur with certain tissue reactions other than tumor necrosis. Necrotizing agents such as croton oil(7), chronic inflammatory agents such as Freund's adjuvant(12), or tumor in an air pouch(7) may have a common denominator of host response to the necrotic debris as the essential preparatory mechanism for PPLO arthritis. The nature of the significant components of necrotic tissue, the cellular and humoral aspects of host response and the favorable conditions for PPLO localization and growth in articular structures are unknown.

Summary. Sporadic polyarthritis in rats observed during spontaneous and induced regression of a lymphosarcoma has been investigated. Cultural studies of the involved periarticular tissues and the tumor yielded PPLO, *Mycoplasma arthritidis*. The respective roles of PPLO, tumor-necrotizing bacterial polysaccharide, and tumor regression in production of the polyarthritic lesions have been evaluated. Arthritis occurred only in PPLO-infected rats with concurrent or prior necrosis of tumor tissue. Bacterial polysaccharide and viable tumor were not essential.

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