Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 106 APRIL, 1961 No. 4

SECTION MEETINGS

DISTRICT OF COLUMBIA	
Georgetown University	February 2, 1961
ILLINOIS	
University of Illinois Medical School	March 14, 1961
IOWA	
State University of Iowa	January 31, 1961
MICHIGAN	
University of Michigan	January 11, 1961
SOUTHERN CALIFORNIA	
University of California, Los Angeles	January 20, 1961

Mycoplasma (PPLO) and Murphy-Sturm Lymphosarcoma.*† (26439)

E. VIRGIL HOWELL, WERNER K. OTTO AND RUSSELL S. JONES
Department of Pathology, University of Utah College of Medicine, Salt Lake City

During the regression of the Murphy-Sturm lymphosarcoma in the rat, a sporadic polyarthritis was found to be due to mycoplasma or pleuropneumonia-like organisms (PPLO)(1). These filterable microorganisms were isolated from the intact and necrotic tumor tissue as well as the joint lesions. Polyarthritis followed intravenous injection of the Mycoplasma in suitable rats. Tetracycline therapy rendered the lymphosarcoma free of PPLO and the sporadic polyarthritis no longer occurred. Jasmin(2) produced PPLO polyarthritis in rats with exudate from air pouches containing the Murphy-Sturm lymphosarcoma and demonstrated PPLO in other tumors(3). PPLO have been found in various cell cultures *in vitro*(4,5). Because of the variable pathogenic effects of PPLO in tissues and cell cultures, it became important to evaluate the role of these microorganisms in regression of the Murphy-Sturm lymphosarcoma. The present study concerns the experimental infection of the tumor, effects of PPLO upon growth rate of the tumor and upon induced and spontaneous regression of the tumor.

Materials and methods. A PPLO-infected tumor was obtained by intravenous injection of PPLO into a rat bearing a PPLO-free Murphy-Sturm lymphosarcoma. The injected PPLO had been isolated recently from an arthritic lesion and grown in Difco PPLO broth. PPLO were cultured regularly from the subsequent transplants of the infected tumor in young, male Holtzman rats, each

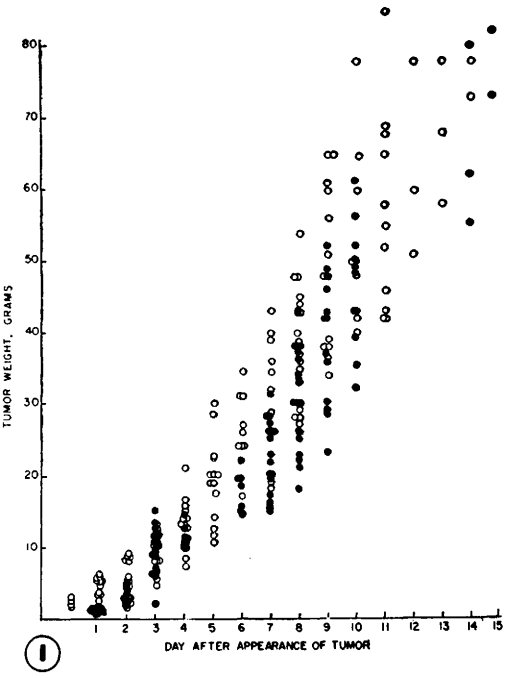
* Presented in part at meeting of Am. Soc. Exp. Biol. and Med., Chicago, April, 1960.

† Supported by grant-in-aid from N.C.I., N.I.H.

weighing 100 to 120 g at time of tumor transfer. Polyarthritis did not occur during the growth of tumor. The tumor infected with PPLO and the tumor free of PPLO were transplanted by subcutaneous injection of lymphosarcoma cells as previously described (6). After the appearance of the tumors mean diameter was recorded daily and the weight derived from a plot of known diameter to mass.

In some groups of rats, cells from a PPLO-infected tumor were injected in one lumbar area and cells from a non-infected tumor in the opposite side. Rats with these double tumors were observed for "spontaneous" regression and for regression produced by *K. pneumoniae polysaccharide* (6). Both of the double tumors were cultured for PPLO. Localization of PPLO in single uninfected tumors was demonstrated by intravenous injection of recently isolated microorganisms into rats before, during and after subcutaneous transplant of tumor cells. About 12 days after tumor transplant, when the tumor had attained mean diameters of 50 to 60 mm, the rats were killed and tumor tissue cultured on PPLO-agar plates (1). PPLO colonies were detected by placing a stained cover slip upon an agar block (7). Groups of 20 or more rats with single tumors were observed for spontaneous regression of the tumor and for induction of regression by injection of polysaccharide from *K. pneumoniae*. To detect any increased susceptibility of the infected tumor, a relatively non-toxic bacterial polysaccharide was prepared by repeated alkaline extraction (8). In some groups, cortisol in an aqueous vehicle, 1 mg/100 g body weight was injected subcutaneously beginning the day of the first of 4 polysaccharide injections and continuing for 6 days thereafter. Sterile, uninoculated PPLO broth, 0.5 ml/100 g body weight, was injected into other groups of rats with PPLO-free or PPLO-infected tumors.

Results. The presence of PPLO in the tumor transplant did not alter the subsequent growth rate of the tumor (Fig. 1), increased the low incidence of spontaneous regression and doubled the regression induced by the bacterial polysaccharide (Table 1). Even intravenous injection of uninoculated broth and presence of established PPLO polyarthritis increased the regression of the PPLO-



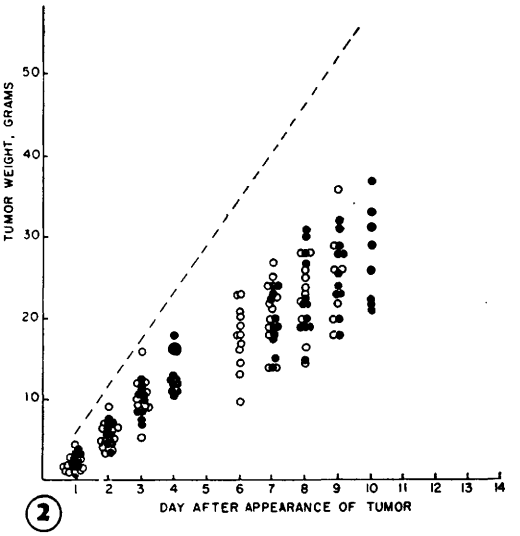


FIG. 1. Growth in representative rats of PPLO-infected (●) and PPLO-free (○) lymphosarcomas beginning at time of tumor appearance, 6 to 7 days after transplant.

FIG. 2. Similar growth rate of 2 lymphosarcomas, PPLO-infected (●), and a PPLO-free (○), in the same rat; combined avg wt of both tumors, - - - -.

TABLE I. Effect of PPLO in Transplanted Lymphosarcoma upon Spontaneous and Induced Regression.

Tumor	Untreated controls	Established polyarthritis*	Sterile broth†	Polysaccharide‡
PPLO-free	6/193 (3.3%)	2/16 (12.5%)	1/19 (5.3%)	3/14 (21.4%)
PPLO-infected	10/182 (5.5%)	8/18 (44.4%)	7/20 (35.0%)	8/17 (47.1%)

* Virulent PPLO inj. 11 days before tumor transplant.
† On 5th day after tumor transplant.
‡ 8th through 11th days after tumor transplant.

infected but not of the PPLO-free tumors (Table I).

When injected intravenously 3 days prior to tumor transplant, PPLO did not appear subsequently in the tumor (Table II). When PPLO were injected on day of tumor transplant, 40% of the tumors contained PPLO. All tumors examined contained PPLO when animals were injected on the 5th and 8th day after tumor transplant. Although the groups were small, the presence of PPLO in the tumor was associated with increased spontaneous regression. When PPLO were injected on the 8th day, the rapidly growing tumor (Fig. 1) led to death within the next few days before regression would be expected to occur.

In rats bearing both PPLO-infected and PPLO-free tumors, PPLO were recovered from the originally uninfected tumor after the tumor had attained large size (Table III). While growth rate of each of the double tumors was slower than that of the single tumor (Fig. 1, 2), the combined mass of the double tumors had a growth rate exceeding that of the single tumors (Fig. 2). Both of the double tumors underwent regression simultaneously. Cortisol prevented the tumor regression produced by the bacterial polysaccharide (Table IV).

TABLE II. Polyarthritis, PPLO in Tumor and Tumor Regression in Rats Injected Intravenously with PPLO before, during, and after Tumor Transplant.

	Day of PPLO inj.*			
	−3	0	+5	+8
Tumor regression	1/10	1/10	3/10	0/10
Polyarthritis	11/20	15/27	13/18	3/15
PPLO in tumor†	0/6	5/13	17/17	10/10

* Tumor transplant, day 0.
† Tumor cultured at day 14.

Several interesting aspects of PPLO growth were observed. Agar block preparations of PPLO-infected tumors disclosed several forms of PPLO growth, typical colonies, small colonies, "Tiny" colonies(9). Some tumor cells appeared to survive 5 to 7 days upon the special agar media. From the cytoplasmic zone of the ghost-like outlines of non-viable cells, deeply azurophilic elements arose and penetrated the agar. These structures, superficially resembling the blue-stained PPLO, never attained large size and occurred only following the placement upon agar media of originally intact tissue or tumor cells either with or without PPLO infection. PPLO colonies usually arose from extracellular sites. In the double tumor experiment, PPLO recovered from the originally PPLO infected tumor grew a few days in clonal form then died, whereas PPLO from the originally un-

TABLE III. Transmission of PPLO from Infected to Originally Non-infected Lymphosarcoma in the Same Rat.

Tumor at transplant	Day after transplant 6 to 8	12 to 13
PPLO-free	0/7	8/8
PPLO-infected	7/7	1*/8

* All tumors contained PPLO but clonal organisms died in a few days in all agar plate cultures except one.

TABLE IV. Regression of 2 Lymphosarcomas (Infected and Non-infected) with PPLO in the Same Rat.

	Untreated controls	Sterile broth*	Polysaccharide†	Polysaccharide† plus cortisol
Regression	1/21	0/10	8/10	0/10
Polyarthritis	3/21	1/10	3/10	0/10

* On 11th post-transplant day. † Beginning 11th post-transplant day.

infected tumor continued to grow on the agar plates.

Comment. In the present study PPLO transmitted with the Murphy-Sturm lymphosarcoma during transplantation, only slightly affected spontaneous regression of the tumor. However, tumors containing PPLO were more susceptible to regression upon intravenous injection of bacterial polysaccharide, sterile broth or presence of polyarthritis due to PPLO. The 2 injected materials and the polyarthritis could act through the same mechanism or mechanisms in producing tumor necrosis, with presence of PPLO in the tumor increasing its susceptibility to the substances mediating the necrosis. These observations do not afford evidence for or against several possible mechanisms of tumor necrosis such as non-specific activation of cytotoxins, stimulation of available iso-antibodies through release of materials from the tumor, or interference with critical metabolic requirements of the tumor. The presence of PPLO did not produce significant histologic or cytologic changes in the lymphosarcoma, although, grossly, such tumors showed more surrounding edema and hyperemia. These minor changes in tumor were in marked contrast to the severe chronic suppurative polyarthritis produced by PPLO isolated from the tumor and injected into normal rats.

In evaluating the role of PPLO upon the tumor several important aspects concerning PPLO were not resolved: a) the number in tumor tissue, b) intra- or extracellular location, c) virulence, d) modification of host "resistance" by large, rapidly growing tumor, and e) anti-PPLO antibody reactions. The turbidity method of counting this particular PPLO was unsatisfactory; broth cultures were often non-turbid but contained the most virulent PPLO as well as numerous PPLO as estimated by placing centrifuged sediment of broth culture upon agar, staining and observing as 2000 magnification. Plate counts were considered inaccurate because of the occasional diffuse spread of organisms and variations in clonal morphology. In unpublished studies we have interconverted virulent and non-virulent PPLO *in vitro*. Upon addition of sterile tumor extract or other materials non-virulent PPLO produce polyarthritis upon intravenous injection in normal rats. Similarly, when non-virulent PPLO were injected into tumor-bearing rats, pathogenic PPLO were recovered from the tumor. These as well as Jasmin's observations(2) indicate that the Murphy-Sturm lymphosarcoma was an excellent environment for growth of pathogenic PPLO. In the present study polyarthritis was readily produced either in normal rats or rats with growing tumor by special cultural enrichment or by maintaining the PPLO in tumor tissue. In other studies, using PPLO of varying virulence, we have confirmed that rats with growing lymphosarcoma were most resistant to PPLO polyarthritis(2,10) while rats with necrotic or regressing tumors were the most susceptible(1). The failure of PPLO to appear in tumor transplanted three days after intravenous injection of PPLO may be explained by the rapid death of PPLO in animal tissues other than synovial structures. The transmission of PPLO from an infected to an originally uninfected tumor in the same animal (Table III) was in contrast to the lack of tumor infection with prior PPLO polyarthritis (Table II). Thus, on one hand, PPLO established in either intact tumor or

joint did not appear in the other tissue site, while, on the other hand, PPLO in one tumor were transmitted to another tumor. Furthermore, the varied time of appearance of separate arthritic lesions suggest non-contiguous dissemination from one joint to another. To the uncertain roles of local suppuration and of the anti-PPLO antibodies in transmission of PPLO in the above circumstances, one may add the role of tumor tissue *per se*—a remarkable milieu in which anigen-antibody and inflammatory responses, quite unlike those in other tissues, would not be unexpected.

Summary. Mycoplasma (PPLO) transmitted with the Murphy-Sturm lymphosarcoma at time of transplant, a) did not modify tumor growth, b) mildly increased the low incidence of spontaneous regression of the tumor and, c) increased the regression due to bacterial polysaccharide and to established PPLO polyarthritis. Following their intravenous injection, PPLO were readily cultured from tumor tissue. When injected prior to transplant of tumor cells, persistent polyarthritis developed but PPLO did not appear in the subsequent tumor. However, when 2 tumors, one infected and one free of PPLO, were transplanted to the same rat, PPLO appeared in the original PPLO-free tumor after moderate tumor size was attained. Agar plate cultures of PPLO indicated several colony sizes and an occasional diffuse surface spread.

1. Howell, E. V., Ward, J. R., Jones, R. S., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 210.
2. Jasmin, G., Richer, C. L., *Experientia,* 1959, v15, 329.
3. Jasmin, G., *Rev. Canad. Biol.,* 1956, v15, 107.
4. Collier, L. H., *Nature,* 1957, v180, 757.
5. Hayflick, L., Stinebring, W. R., *Ann. N. Y. Acad. Sci.,* 1960, v79, 433.
6. Thomas, C. R., Jones, R. S., *Arch. Path.,* 1960, v70, 441.
7. Dienes, L., Ropes, M. W., Smith, W. E., Madoff, S., Bauer, W., *N. Eng. J. Med.,* 1948, v238, 509.
8. Jones, R. S., Carter, Y., *Arch. Path.,* 1957, v63, 484.
9. Shepard, M. C., *J. Bact.,* 1956, v71, 362.
10. Jasmin, G., *Ann. Rheum. Dis.,* 1957, v16, 365.

Received January 30, 1961. P.S.E.B.M., 1961, v106.

Comparison of Autologous, Homologous, and Heterologous Normal Skin Grafts in the Hamster Cheek Pouch.* (26440)

STANLEY N. COHEN† (Introduced by J. R. Brobeck)
Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia

During recent years, the cheek pouch of the Syrian hamster has become an increasingly popular site for transplantation of fetal (1,2), malignant(3,4,5,6), and normal adult (7) tissues. Toolan(8) has observed increased growth potentialities of normal heterologous tissues implanted into the cheek pouch of the cortisonized hamster. Lemon, *et al.*(3) have reported the persistence of some normal human tissues in the cheek pouch of untreated hamsters but have failed to observe definite evidence of growth. It appeared desirable to evaluate carefully the cheek pouch as a site for grafted tissues and to compare the behavior of normal autologous, homologous, and heterologous grafts in both cortisonized and unconditioned hosts.

Methods. Male young adult Syrian hamsters, from a non-inbred colony, weighing between 70 and 100 g were used in all of these experiments. The animals were implanted with 1 mm square portions of normal skin according to the method of cheek pouch implantation described by Lutz, *et al.*(9). The entire thickness of abdominal skin was taken for the hamster homografts and autografts. For the heterografts, a 1 mm thickness of

* Supported in part by U.S.P.H.S. grants.

† Present address: Mount Sinai Hospital, New York.