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Treatment of Leukemic (L-1210) Mice with Double-Stranded Polyribonucleotides (33503)

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Many animal leukemias are induced by viruses, and human leukemia may also be a viral etiology; therefore, inhibition of viral multiplication has been attempted in order to treat leukemia. In a recent paper Wheelock and Larke (1) reported on the efficacy of interferon in prolonging the life span of mice with established Friend virus leukemia. The authors also demonstrated that injection of Statolon (an interferon-inducing substance) prolonged the life of mice with established leukemia. Wheelock (2) had previously shown that some nontumorogenic viruses (which can induce interferon) inoculated into mice prior to the Friend leukemia virus could prolong the life of the mice.

Among the most potent inducers of interferon are the double-stranded polyribonucleotides described by Hilleman and co-workers (3). These polyribonucleotide complexes can induce high levels of circulating interferon and thus protect animals from lethal viral infections.

The present communication describes experiments in which the life span of leukemic (L-1210) mice is prolonged by administration of polyribocytidylic acid: polyriboinosinic acid (1:1) copolymers.

Materials and Methods. (i) *Mice.* Female BDF_1 mice, 18–20 g were obtained from Jackson Memorial Laboratories.

(ii) *L-1210 leukemia.* Leukemic mice were obtained from Dr. I. Wodinsky, Arthur D. Little, Inc., and maintained in an ascitic form, by weekly transfer of 10^6 cells intraperitoneally (i.p.) in BDF_1 mice.

(iii) *Dougle-stranded polyribonucleotides.* Polyribonucleotides were products of Miles Laboratories, Inc. Dougle-stranded complexes of polyinosinic acid and polycytidylic acid (1:1) [poly I:poly C] were prepared by dissolving equal weights of each polynucleotide in 0.15 *M* NaCl at a concentration of 10 mg/ml. The solutions were heated to 80° mixed thoroughly and kept at 80° for 15 min. The solution was then allowed to cool slowly to room temperature over a period of 3 hr. An alternate solvent for the polynucleotides was 0.5 *M* NaCl–0.02 *M* citrate–0.01 *M* Tris, pH 7.3–0.001 *M* EDTA. Two batches of poly I:poly C were prepared similarly; however, slightly varying results were obtained.

(iv) *Inoculation of BDF_1 mice.* Groups of 10 mice were inoculated with 10^6 L-1210 ascites cells i.p. The polynucleotide complex was given i.p. unless otherwise mentioned. The standard deviation and percentage increase in life span were calculated by published procedures (4).

Results. Multiple injections of poly I: poly C prolonged the life span of leukemic mice. Control mice died 7.5–8 days after inoculation, with a standard deviation ranging from 0.65 to 0.81 in different experiments. The mice treated with 5 mg/kg poly I: poly C lived 25–40% longer than control mice. Doses of poly I:poly C above 10 mg/kg were toxic resulting in early deaths of the mice. The results are given in Table Ia.

Mice treated with a single dose of poly I:poly C at 5 or 20 mg/kg 1 day prior to inoculation with L-1210 cells showed an insignificant increase in survival time. Thus, the effect does not seem to be simply an increase in nonspecific host-resistance.

TABLE I. Prolongation of Life in Mice Inoculated with L-1210 Cells on Day 0 by Treatment with Poly I:Poly C.

Dose (mg/kg)	Injection schedule	Increase in Life Span (ILS) (%) Batch I	Batch II
a			
5	Days −1 to 7	43, 46	ND[a]
10	−1 7	8	ND
20	−1 7	Toxic	ND
b			
5	−4 0	ND	21
	−1 7	46	21
	0 7	54	21
	1 7	39	28
c			
1.25	1 7	ND	21
2.5	1 7	ND	29, 27
5	1 7	ND	29, 20
7.5	1 7	ND	27
10.0	1 7	ND	27

[a] ND = not done.

The effect of various treatment schedules was compared at 5 mg/kg of poly I:poly C. The data indicate (Table Ib) that the different treatment schedules gave similar increases in life span.

The effect of different levels of poly I:poly C given in a multiple dosage regiment is shown in Table Ic. There is not a good dose–response relationship; however, 5 mg/kg seemed to be the useful dose on the basis of response and toxicity.

The individual polyribonucleotides as well as complexes of several other polyribonucleotides were all inactive. Poly I:poly C was active when administered i.p. or subcutaneously (s.c.) but was inactive when given orally (Table II) on Days 1–7.

Discussion. The results demonstrate the effect of treatment of the host with double-stranded poly I:poly C on prolonging life span of leukemic mice. The effect of the polynucleotide complex is not a direct cytotoxic or sytostatic effect on the L-1210 cells, since in cell culture 83 μg/ml of poly I:poly C has no effect on cell growth. Also when poly I:poly C (1 mg/ml) is mixed *in vitro* with L-1210 ascites cells and the cells are washed and reinjected into mice, there is no prolongation of life. This would agrue that the poly I:poly C is causing the host to produce a material which is cytostatic.From the work of Hilleman on interferon production by double-stranded polynucleotides (3) and the work of Wheelock (1) showing the antileukemic effect of interferon, it is logical to assume that the active material is interferon. Other interpretations are indeed possible since the polynucleotides have been shown to have adjuvant properties and hasten appearance of antibodies (5); thus, possibly an antibody response to the L-1210 inoculation is being produced which slows the growth of the cells. This latter postulation is not as attractive since the adjuvant activity of the polynucleotides is seen when the material is injected with or prior to the antigen, while the effects seen in this paper indicate that the poly I:poly C even works after the inoculation of L-1210 cells.

Application of these results to the human leukemias would be speculative; however, it is of interest that synthetic polymers now in test for antitumor activity induced low levels of circulating interferon (6).

Summary. The BDF_1 mice when injected with 10^6 L-1210 ascites leukemia cells die in 8 days. When treated from days 1 to 7 with 5 mg/kg of polyinosinic acid:polycytidylic acid (1:1) they have a 25–40% increase in life span. The material is effective when given

TABLE II. Effect of Other Polynucleotide Complexes on L-1210 Leukemic Mice.

Dose (mg/kg)	Compound	Route of administration days 1–7	ILS (%)
5	Poly I	i.p.	0
	Poly C	i.p.	0
	Polyguanylic:polycytidylic (1:1)	i.p.	7
	Polyadenylic:polyuridylic (1:1)	i.p.	0
10	Poly I:poly C[a]	i.p.	21
	Poly I:poly C[a]	s.c.	21
	Poly I:poly C[a]	oral	0

[a] Batch II.

i.p. or s.c., but not by oral route, and the component polyribonucleotides are also inactive. The activity of the double-stranded complex in prolonging survival is not due to *in vitro* cytotoxic or cytostatic activity. It is proposed that the activity of poly I:poly C is due to the induction in the host on interferon which then slows the multiplication of the L-1210 cells *in vivo*.

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Host Specificity and Serologic Disparity of African Swine Fever Virus and Amphibian Polyhedral Cytoplasmic Viruses (33504)

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African swine fever virus (ASFV) and an amphibian-pathogenic virus, designated frog virus 3 (FV3), display remarkable similarity when examined by electron microscopy (1–4). Both viruses contain DNA, multiply in the cytoplasm, have a similar mode of egression from infected cells, and are inactivated by lipid solvents (1, 4, 5). The number of subunits that comprise the capsids of either virus, however, has not yet been ascertained.

The similarity of these agents prompted studies to determine if ASFV and the polyhedral cytoplasmic viruses observed in frogs (2), bullfrogs (6), and newts (7) have a similar host range or antigenic relationship. All of the isolates from amphibia appear to be serologically related (8, 9). Burton *et al.* (10) reported that an amphibian may serve as a host for a virus that is pathogenic for a mammal (Western equine encephalitis). Thus, we investigated the susceptibility to ASFV of an amphibian host known to be susceptible to the polyhedral cytoplasmic viruses isolated from amphibia (6, 9). No reservoir in nature, other than the swine, is known for ASFV. These studies also permitted serologic analysis of these morphologically similar agents. Knowledge relevant to animal-host reservoirs or serologic relationships would have epidemiologic significance and taxonomic interest as these two viruses, as well as the DNA containing, polyhedral, lymphocystis virus of fish and Tipula and Sericesthis iridescent viruses of insects do not fit into the present virus classification scheme.

Materials and Methods. Animals. Tamworth pigs, weighing approximately 60 lb were used throughout. Toads, *Bufo fowleri,* approximately 2.5 cm long, were from the shore of Lake Erie. The toads were maintained in tilted, stainless steel cages containing sand, 1–4 cm deep, and some tap water at

[1] A portion of this work was conducted while the senior author was in the laboratory of Dr. D. H. Moore at Rockefeller University, New York, N.Y..

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