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The Role of Interferon in the Spontaneous Regression of Friend Virus Induced Leukemia[1] (38963)

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The Friend murine leukemia virus (MuLV) induces a progressive erythroblastic leukemia in mice which ultimately leads to death in virtually all of the animals inoculated. Several years ago, we described the isolation of a stable variant of Friend MuLV, indistinguishable from the conventional strains of virus, which induces a leukemia that spontaneously regresses in a large proportion of the infected animals (1, 2).

Wheelock *et al.* (3) subsequently reported that the leukemia caused by conventional strains of Friend MuLV could be induced to regress by treatment of the animals with Statolon. These authors further suggested that Statolon induced regression is not due to interferon, since other interferon inducers did not have this effect (4).

Since the mechanisms involved in the natural regression of leukemia are as yet unclear, it was of interest to determine the effect of Statolon and Poly-IC, and the role of interferon in spontaneous regression of leukemia.

Materials and Methods. Virus. Stocks of the conventional (CFV) and the regressing strains of Friend MuLV (RFV) were prepared as 20% cell-free spleen homogenates from leukemic animals, as previously described (5). Vesicular stomatitis virus (VSV), Indiana strain, was grown in mouse L cells and titrated by plaque assay in these same cells as described below.

Animals. Random bred male Swiss/ICRHa weanling mice from our colony were used in the experiments described. The animals were inoculated with virus stocks intraperitoneally and palpated two or three times weekly to monitor for leukemia development and regression. We have previously shown that palpation for splenomegaly is an accurate reflection of spleen weight and pathology (1, 5).

Interferon assay by plaque reduction. Cultures of mouse L cells were grown to confluence in Dulbecco's modified Eagle's medium containing 15% calf serum. The cultures were incubated with the preparation to be tested, diluted in medium with 5% serum, for 24 hr. The test material was then removed, the cells were rinsed three times with serum-free medium, and approximately 50 plaque-forming units (PFU) of VSV in 0.1 ml medium were added. The viruses were allowed to adsorb for 1 hr, following which, 5 ml of medium containing 5% serum and 0.9% agar was added. The cultures were incubated for 24 hr, stained with neutral red, and scored for plaques 12–24 hr later. Sera were tested both before and after acid treatment (5 days at pH 2, 4°, followed by reneutralization), to confirm that the activity observed was due to interferon.

Interferon assay by yield reduction. Confluent monolayers of mouse L cells were treated with test preparations as described above, washed, and infected with VSV (approx 0.1 PFU/cell). Following incubation for 24 hr, the supernatant was removed, and centrifuged at 400*g* to remove cell debris. The supernatants were titrated by infecting L cells grown in Linbro FB-16-24TC plates. At least six wells were used for each dilution of each supernatant, and the wells were scored for CPE at 24 and 48 hr postinoculation. The titers expressed as $TCID_{50s}$ (tissue culture infectious doses), were calculated by the method of Reed and Muench (6).

Interferon standard. A control titration of mouse reference interferon (Research Resources Branch, National Institute of Allergy

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TABLE I. EFFECT OF STATOLON AND POLY-IC ON THE INCIDENCE OF LEUKEMIA AND LEUKEMIA REGRESSION

Virus stock	Treatment	Incidence of leukemia	Incidence of regression
CFV	PBS[a]	28/28[d]	0/28[e]
	Statolon[b]	20/22	2/20
	Poly-IC[c]	26/30	6/26
RFV	PBS	26/28	10/26
	Statolon	17/20	8/17
	Poly-IC	23/29	8/23

[a] Phosphate buffered saline, 0.2 ml inoculated intravenously at 3 days postvirus inoculation.

[b] 4 mg in 1% $NaHCO_3$, inoculated intravenously at 3 days postvirus inoculation.

[c] 25 μg in saline, inoculated intravenously at 3 days postvirus inoculation.

[d] Number of mice leukemic/number of mice at risk.

[e] Number of mice regressed/number of mice leukemic.

TABLE II. TITRATION OF INTERFERON ACTIVITY BY YIELD REDUCTION

Test serum	Dilution	Virus yield[a]	Yield reduction (%)
None	—	8.9	—
Interferon standard	1:250	6.8	99.2
	1:1000	8.1	84.2
	1:4000	8.5	60.2
Regressed[b]	1:25	8.6	49.9
	1:100	8.7	37.0
PBS[c]	1:25	8.7	37.0
Statolon[d]	1:50	7.7	93.7
	1:200	8.0	87.4

[a] Log $_{10}$ $TCID_{50/ml}$ of culture supernatant.

[b] Pooled serum from RFV infected mice whose leukemia had regressed.

[c] Pooled serum from control mice inoculated intravenously with 0.2 ml PBS and bled 6 hr later.

[d] Pooled serum from control mice inoculated intravenously with 4 mg Statolon and bled 6 hr later.

and Infectious Diseases, Catalogue Number G002-904-511) was included with each series of determinations.

Statolon and Poly-IC. Statolon was obtained through the courtesy of Dr. R. J. Hosley, Eli Lilly and Company, Research Laboratories, Indianapolis, Indiana. Poly-IC (double stranded polyinosinic acid-polycytidilic acid) was purchased from P-L Biochemicals, Milwaukee, Wisconsin.

Results and Discussion. Animals inoculated with CFV develop splenic leukemia which only very rarely regresses spontaneously. Treatment of CFV infected, leukemic mice with either Statolon or Poly-IC induced a 10 and 23% incidence of leukemia regression, respectively (Table I). That Poly-IC was more efficacious than Statolon, in contrast to the result reported by Wheelock *et al* (4), may reflect the use of different strains of virus and test animals in the two studies.

Neither Statolon nor Poly-IC treatment had any significant effect on the spontaneous regression normally observed in RFV infected leukemic mice. The spontaneous regression in PBS treated control animals was within the limits which we have observed (30–70%) in several thousand animals inoculated with various stocks of RFV. The time parameters for the occurrence of leukemia and regression were the same in all of the groups.

No significant effect of Statolon or Poly-IC on the incidence of leukemia in RFV or CFV-infected mice was observed (Table I).

To determine its role in the spontaneous regression of leukemia, assays of interferon activity in sera from regressed mice were made using both the plaque reduction test and the more sensitive yield reduction test. No significant amounts of interferon were detected in these regressed sera, above that observed in control sera (Fig. 1, Table II). These mice were, however, competent to generate high levels of circulating interferon in response to the inducers Statolon or Poly-IC (Fig. 1, Table II).

To determine whether CFV or RFV themselves were capable of inducing interferon activity, sera of mice were tested at 6, 30, and 50 hr postvirus inoculation. No significant activity was detected above that in control sera. In addition, sera from RFV induced leukemic mice at 28–35 days postvirus inoculation, the time just prior to when spontaneous regressions begin to occur (1, 2), did not contain any significant interferon activity.

We conclude, therefore, that since re-

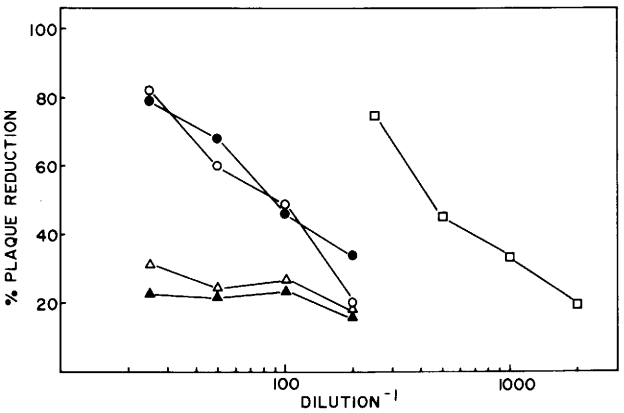

FIG. 1. Assay of interferon activity by plaque reduction. (○—○) Serum from Statolon treated mice; (●—●) serum from Poly-IC treated mice; (△—△) serum from mice in which leukemia had spontaneously regressed; (▲—▲) serum from control mice; (□—□) interferon standard.

gressed serum does not contain higher levels of interferon than controls and that interferon inducers do not affect the incidence of regression under conditions in which circulating interferon is induced, that interferon does not play a significant role in the spontaneous regression of Friend virus leukemia.

Summary. The spontaneous regression of leukemia induced by RFV (the regressing strain of Friend MuLV) does not involve interferon. Inducers of interferon do not affect regression. Interferon activity in sera from infected or regressed animals is the same as that found in control sera.

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