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Interferon and Murine Leukemia. II. Factors Related to the Inhibitory Effect of Interferon Preparations on Development of Friend Leukemia In Mice. (31673)

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In the preceding communication we presented the results of experiments demonstrating an inhibitory effect by interferon preparations on the development of Friend leukemia in mice. We present here an analysis of various factors pertaining to the demonstration of this phenomenon.

Materials and methods. The viruses employed and their methods of assay, the techniques of cell culture, the preparation of interferon and its assay, the different strains of mice employed, have all been described in the preceding communication.

In some interferon assays a tube dilution technique was also employed. Two-fold dilutions of interferon in nutritive medium were incubated with L cells for 18-24 hours at 37°C. The medium was then removed, the cell cultures washed once with PBS, and medium containing 100 $TCID_{50}$ of vesicular stomatitis virus (V.S.V.) was added. The interferon titer was expressed as the highest dilution which protected more than 75% of the cell sheet at a time when complete cell destruction was observed in control cultures.

Diffusion of interferon after intraperitoneal (i.p.) and intravenous (i.v.) inoculation of interferon. Mice were inoculated i.p. with 0.25 ml of an interferon preparation. At intervals thereafter 2.25 ml of PBS were inoculated i.p. and light pressure applied to the abdomen to ensure dispersion of the fluid. After brief exposure to ether, the mice were exsanguinated by section of the carotid vessels and the blood collected for assay of serum interferon. The abdominal fluid was aspirated and centrifuged at 1500 rpm for 10 minutes to sediment the peritoneal macrophages. The supernatant was stored at −20°C prior to interferon assay. Spleen, kidney and liver to be tested for interferon were washed twice in 20 ml of PBS and frozen immediately in dry ice(1). A 10% (wet weight) tissue suspension was prepared in PBS, treated at pH 2, centrifuged at 110,000 *g* for 1 hour, and the pH of the supernatant adjusted to 7.

Results. Capacity of Friend virus to induce interferon. To determine whether Friend virus induced the formation of interferon, Swiss and IC mice were inoculated i.v. with 0.2 ml of undiluted virus (log 3.5 SD_{50}/ml). Equivalent numbers of mice were inoculated with NDV (log 9.0 PFU/ml) (positive control). Small quantities of an interferon-like substance (titer 1:10-1:40) were detected in both the serum and spleen of Swiss and IC mice, 24 but not 5 hours after inoculation of Friend virus.*

Large quantities of interferon were detected in mice inoculated with NDV (5 hours, 1:5120; and 24 hours 1:1280 in serum and spleen).

* The imprecise *in vivo* assay of Friend virus renders quantitative studies difficult. Thus the limited interferon response to the i.v. inoculation of Friend virus may reflect an inadequate(2) number of virions inoculated rather than an intrinsic property of the virus.

To determine whether interferon was present in the tissues of mice with Friend disease, other mice were sacrificed 7, 14, 21, 28, 35, 37 and 45 days after inoculation of Friend virus. All mice had significant splenomegaly. Each serum and spleen was assayed for the presence of interferon at a 1:6 or 1:10 dilution. No inhibition of the challenge virus was observed.

Although Friend virus did not appear to induce the production of significant amounts of interferon, the absence of this factor in the tissues of leukemic mice may also have been explained by a) the presence of a substance(s) in leukemic mice which inactivated interferon, or b) an inability of leukemic mice to produce interferon.

To test the first hypothesis, 8 units of interferon were incubated for 1 hour at room temperature and then for 18 hours at 4°C with a 1:5 dilution of serum from leukemic mice. No inhibition of interferon activity was observed.

To test the second hypothesis, mice infected with Friend virus 3 and 6 weeks previously, were inoculated i.v. with NDV (log 8.3 PFU). There was no significant difference between leukemic and normal mice in the interferon response to inoculation of N.D.V. (see also 3).

Absence of detectable neutralizing antibody to interferon in mice with Friend disease inoculated daily with preparations of interferon. In the experiments described in the previous communication, Swiss mice were inoculated daily or twice daily with potent interferon preparations. It was of interest to determine whether these mice formed detectable neutralizing antibody to interferon.

In 2 experiments, sera were obtained from mice inoculated twice daily (0.2 ml) for 4 weeks with an interferon preparation titering 1:67,500/2 ml and for 6 weeks with a preparation which titered 1:12,800/2 ml. In each experiment sera were pooled (not heat inactivated) and incubated either at a 1:5 or 1:8 dilution for 1 hour at room temperature and then for 18 hours at 4°C with either 2½ or 8 units of interferon (as determined by simultaneous titration of interferon). Sera from normal and leukemic mice not inoculated with interferon were included in the tests. No significant anti-interferon-like activity was detected in any of the sera tested.

Diffusion of interferon after intravenous and intraperitoneal inoculation. In the preceding communication it was shown that interferon preparations inoculated intraperitoneally inhibited the development of splenomegaly in Friend disease, whereas interferon preparations inoculated intravenously proved ineffective. Since pharmacologic effects may depend on route of administration, the diffusion of interferon after intravenous and intraperitoneal inoculation was investigated. The titer of interferon in the serum decreased markedly after intravenous inoculation (Table I). Significant quantities of interferon were recovered in the urine.† Total urine could not be measured with complete accuracy and only the urinary interferon concentration is presented (Table I).

In contrast to the rapid disappearance of interferon from the serum after i.v. inoculation, interferon inoculated intraperitoneally decreased at a much slower rate and was still present 5 hours after inoculation (Exp. 2, Table I). Moreover, interferon was detected in the serum at hourly intervals and a relatively constant level was maintained. The serum interferon titer could be increased by increasing the interferon concentration within the peritoneal cavity (Exp. 3, Table I) and at this increased serum level, interferon was also detected in extracts of spleen, kidney and lung.

Discussion. Interferon was not detected in the tissues of mice infected with Friend virus in our experiments, nor was it recovered from the spleens of Balb/c mice infected with Rauscher virus in the experiments of Peries and his coworkers(4). There are several other *in vivo* systems in which interferon was present shortly after viral infection, and was not detected thereafter in spite of continued viral multiplication(5,6). In these instances it has been suggested that interferon continues to be synthesized by infected cells but is not liberated by these cells(6). One may also

† It was calculated that approximately 0.1-05% of the total interferon inoculated i.v. was excreted in the urine.

TABLE I. Diffusion of Interferon in Swiss Mice.

Hours after inoculation	Inoculated intravenously — Titer of interferon† — Exp 1 — Serum‡	Urine	Spleen‖	Exp 2 — Serum§	Urine	Inoculated intraperitoneally — Titer of interferon† — Exp 1 — Periton.	Serum	Exp 2 — Periton.	Serum	Spleen	Exp 3 — Periton.	Serum	Spleen	Kidney‖	Lung¶
1/60	3840	—	8	480	<50	4800	<15	12800 9600	<15 NT	<10	128,000	<10	<8	<8	<10
1/4	—	—				4800* 6400	30 40								
1/2	240	—	64	40	800			4800 4800	30 13	<10 <10	32,000 48,000	264 90	12	12	15
1.	80	>640	24	<20		2400 1200	60 60	1600 2400	30 30	<10 <10	24,000 32,000	156 90	16	16	<10
2.	10	800	<8					800 600	30 20	<10 <10					
3.	<10	80	<8			200 150	20	600 150	11 20	<10 <10					
4.	<10	30	<8												
5.						50 <50	<12 <20	100 200	13 <15	<10 <10					

* Two values indicate results obtained from 2 mice tested individually.
† Titers of interferon/ml expressed as reciprocal of the dilution.
‡ 0.2 ml of an interferon preparation titering 1:64,000/ml inoculated i.v.
§ 0.2 ml of an interferon preparation titering 1:16,000/ml inoculated i.v.
‖ Dilution of a 10% (wet wt) suspension.
¶ Dilution of a 2% (wet wt) suspension.

speculate that the simultaneous presence in tissues of factors antagonistic to interferon (stimulons(7)) may mask the detection of interferon in the usual assay systems.

Whatever the explanation, the absence of detectable interferon in mice with Friend disease and the failure to detect factors inactivating interferon in the sera of these mice suggested the feasibility of administering interferon in an attempt to influence the evolution of the disease. Mice tolerated repeated i.p. inoculations of concentrated preparations of interferon for 4-6 weeks and did not produce detectable neutralizing antibody to interferon. The results of our experiments suggest that the i.p. route of administration may be superior to the i.v. route, if the maintenance of an interferon level in the serum is important in inhibiting viral multiplication(8). Thus after intraperitoneal inoculation, the amount of interferon in the peritoneal cavity decreased gradually and a significant level of interferon was maintained in the serum for several hours, suggesting a continuous diffusion of interferon into the circulatory system. In contrast the level of interferon in the serum decreased markedly after intravenous inoculation (see also 1,9) It was of interest to note that interferon was recovered in the urine.

These findings on the diffusion of interferon may bear on the inhibitory effect of interferon preparations observed after i.p. inoculation of mice (preceding communication) and the inefficacy of these preparations when inoculated intravenously.

Summary. Various factors pertaining to the absence of interferon in the tissues of mice with Friend disease and the passive administration of interferon to mice with Friend disease have been investigated.

The diffusion of interferon after intravenous and intraperitoneal inoculation has also been described. After intravenous inoculation the titer of interferon in the serum decreased markedly and interferon was recovered in the urine. Interferon inoculated intraperitoneally diffused from the peritoneal cavity into the circulation at a relatively constant rate and a significant level of interferon was maintained in the serum for as long as 5 hours.

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