with serotype II or III foamy virus antigens. Analysis of serologic and virus-isolation data obtained over a 4-year period revealed that approximately 50% of the rhesus monkey sera tested were CF-positive for type I foamy virus and 16% were positive for SV_{40}. With grivet monkeys, approximately 60% were CF-positive for type II foamy virus, and none were positive for SV_{40}.

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Efficacy of Interferon in the Treatment of Mice with Established Friend Virus Leukemia* (32663)

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On the basis of the possible virus etiology of human leukemia, treatment of this disease might logically be considered in terms of virus inhibition, perhaps by employing the principles of viral interference. Such an approach was attempted in an earlier study (1) in which the sequential inoculations of six virus types into a patient with acute myelogenous leukemia were followed by transient clinical and hematological remissions. Since the mechanisms involved in the production of these remissions could not be determined with certainty, a murine system was developed in which the effects of nontumor viruses on virus-induced leukemia could be more readily investigated.

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In earlier studies (2,3) it had been found that Sendai virus (parainfluenza 1) inoculated into mice *prior* to Friend leukemia virus could markedly inhibit the replication of and the splenomegalic response to Friend virus and prolong the life of leukemic mice. Since human leukemia, be it of virus etiology or otherwise, presents as an established disease, it was of interest to study the effects of Sendai virus in mice with *established* Friend virus leukemia.

The present communication describes experiments in which the life of mice with established Friend virus leukemia was prolonged following inoculation of Sendai virus. As evidence suggesting that interferon is the mechanism involved in this prolongation of life, it will be shown that the repeated administration of interferon or an injection of Statolon (an interferon-inducing substance

present in extracts of *Penicillium stoloniferum*) (4) also prolonged the life of mice with established leukemia. Additional evidence implicating an intereferon mechanism derives from the observation that murine leukemia is suppressed in proportion to the amount of interferon induced by Sendai virus inoculation. These findings may contribute to a broader understanding of the possible application of interferon to the treatment of viral diseases, since previous evaluation of interferon as a therapeutic agent has been based largely on the premise that it acts at an early stage in virus infections and has little effect on established viral disease (5).

Materials and Methods. (1) Mice. Female DBA/2 mice, age 6–8 weeks, were obtained from the Jackson Memorial Laboratories. *(2) Viruses. (a) Friend leukemia virus* (FV) was obtained from Dr. Charlotte Friend in the form of infected DBA/2 mice (6). The enlarged spleens were removed from the mice 3 weeks after infection, and a 20% suspension of them was made in phosphate-buffered saline (PBS). The suspension was then centrifuged at 6000 rpm for 1 hour, and the supernatant fluid was passed through a Millipore filter with a pore size of 450 mμ, quick-frozen, and stored at —70°C. In all experiments described below, mice were inoculated intraperitoneally with Friend virus, 800 leukemia-producing $doses_{50}$ per mouse.

(b) Vesicular stomatitis virus (VSV) was propagated in mouse L-cell monolayer cultures. The infected cultures were sonicated and clarified by centrifugation at 4000 rpm for 30 minutes; the supernatant fractions were then passed through a Millipore filter with a pore size of 450 mμ, quick-frozen, and stored at —70°C.

(c) Sendai virus (SV, obtained from Dr. Fred Davenport) was propagated in the allantoic sac of 10-day-old embryonated chicken eggs. After 2 days' incubation at 37°C, infected allantoic fluids were pooled, clarified by centrifugation, and spun at 105,000 *g* for 7 hours. The pellets were then washed with saline, dispersed in PBS by sonication, filtered as described above, quick-frozen, and stored at —70°C.

(d) Newcastle disease virus (NDV). The Hickman strain was employed. Virus seeds were propagated in the allantoic sac of 11-day-old chick embryos. The infected allantoic fluids were clarified by centrifugation at 2500 rpm for 1 hour and then centrifuged at 105,000 *g* for 6 hours. The pellets were washed with phosphate-buffered saline, dispersed by sonication, passed through a 450-mμ Millipore filter, quick-frozen, and stored at —70°C. The titer of the virus seed employed in these studies was 2×10^9 EID_{50}/ml.

(2) Cell cultures. The L strain of mouse fibroblasts derived from normal mouse skin was employed in the assay for interferon. Growth medium for the L cells consisted of Eagle's minimum essential medium supplemented with tryptose phosphate broth (4%) and fetal calf serum (10%). The concentration of sodium bicarbonate was 1.75 gm/liter, and all cell-culture vessels were gassed with 5% CO_2 in air before incubation at 37°C.

(3) Peritoneal washings. Mice were anesthesized with ether and exsanguinated, and the abdominal wall was shaved. The peritoneal cavity was opened and washed with 5 ml of PBS. The recovered washing was then clarified by centrifugation at 2000 rpm for 5 minutes and processed as described below for the interferon assay. The supernatant fluids from the peritoneal washings of mice inoculated with Sendai virus were acidified to pH 2 for 24 hours, neutralized, and assayed for interferon.

(4) Interferon assay. The specimens to be tested for interferon were diluted serially in growth medium, and 1 ml of each dilution was added to 1-day-old cultures of mouse L cells grown in incomplete monolayers in screwcap tubes. After 20 hours of incubation at 37°C, the cultures were washed once with 4 ml of PBS. To each tube, 1 ml of warm protein-free Eagle's medium was added, and then 1 ml of cold growth medium containing 2000 tissue culture infective doses ($TCID_{50}$) of vesicular stomatitis virus was inoculated. This amount of virus produced gross cytopathic effects in control cultures in 24–30 hours. Cultures were considered to be protected when less than 10% of the cells showed cytopathic effects at a time when more than 75% of control cells exhibited cytopathic effects.

Interferon titers are expressed in culture-protecting units (CPU), that is, as reciprocals

of the highest dilution of the specimen, 1 ml of which protected cultures against challenge with VSV. No specimen was tested at a dilution of less than 1:10.

(5) Preparation of interferon. Mice were inoculated intravenously with NDV $10^{8.8}$ EID_{50} per mouse and, 8 hours later, were exsanguinated, and the sera were separated from clotted blood. The spleens were removed and a 50% extract by weight was made in PBS by grinding in a Servall Omnimixer. The extract was sonicated by a Blackstone 400-W ultrasonic probe at maximum power for 15 sec, and the serum was then added. The preparation was acidified to pH 2 for 48 hours and then spun in an ultracentrifuge at 105,000 *g* for 2 hours. The supernatant fluid was then removed and brought to neutrality.

(6) Inoculations of leukemic mice. The abdominal walls of the mice were shaved and a drop of mineral oil was applied to the skin to visualize the enlarged livers and spleens. Peritoneal inoculations were made in areas of the abdomen devoid of liver and spleen to avoid puncture of these organs. All mice dying within 6 hours after inoculation were examined for gross blood in the peritoneal cavity; if blood was present, they were classified as traumatic deaths and were excluded from the study. All other deaths were attributed to leukemia.

Results. Effect of time of Sendai virus inoculation on survival of mice with Friend virus leukemia. Previous experiments have shown that Sendai virus inoculated together with or as late as 10 days after Friend virus has no inhibitory effect on the splenomegalic response to Friend virus (2). The following experiments were conducted to determine whether Sendai virus could prolong the life of leukemic mice when inoculated after Friend virus. Sets of 25 mice were inoculated intraperitoneally (i.p.) with Sendai virus $10^{8.2}$ EID_{50} per mouse in a 0.2-ml volume on the twentieth or thirtieth day after an inoculation of Friend virus. Leukemic control mice received 0.2 ml of saline on the thirtieth day. The dose of $10^{8.2}$ EID_{50} of Sendai virus had been shown to produce maximum inhibition of the splenomegalic response when inoculated prior to Friend virus (3).

As illustrated in Fig. 1, control mice began

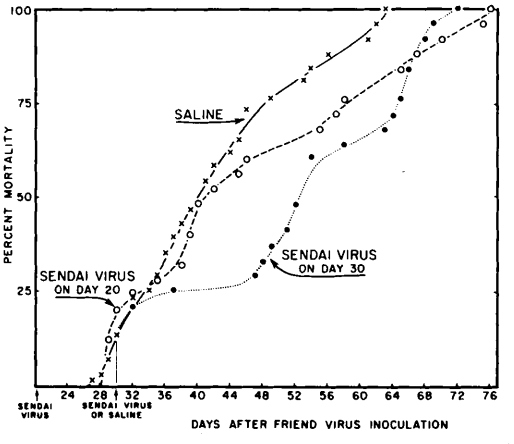

FIG. 1. Prolongation of life of mice inoculated intraperitoneally with Sendai virus on day 20 or day 30 following Friend virus.

to die 27 days after Friend virus inoculation, and all mice were dead on the sixty-third day. Approximately half of the mice inoculated with Sendai virus 20 days after Friend virus died initially at the same rate as the Friend virus controls, and the remainder died an average of 10 days later than the controls. However, mice inoculated with Sendai virus 30 days after Friend virus had reduced death rates as compared with Friend virus controls during the subsequent 12 days, after which time deaths occurred at the same rate in both groups.

The results of this experiment indicate that the administration of Sendai virus can prolong the life of mice with established FV leukemia. Furthermore, there is, in the course of Friend virus leukemia, an interval during which the disease is particularly susceptible to inhibitory effects of Sendai virus; this interval encompasses the period when mice are beginning to die from their leukemic disease.

Production of interferon in leukemic mice after inoculation with Sendai virus. Experiments were performed to determine whether the prolonged survival of leukemic mice which were inoculated with Sendai virus 30 days after Friend virus might be due to interferon production. Interferon production in Friend virus-infected mice in response to Sendai virus inoculation has been described in part previously (2). Briefly, it was found that mice undergo a marked reduction in the interferon response to Sendai virus when the latter is ad-

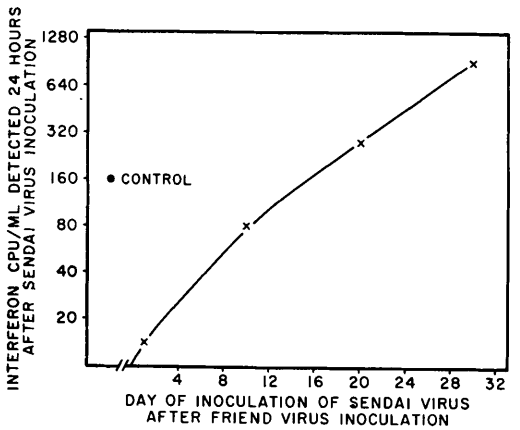

FIG. 2. Interferon production in mice inoculated with Sendai virus at intervals following Friend virus. Titers of interferon represent the amount detected in peritoneal washings 24 hours after Sendai virus inoculation.

ministered i.p. 1–10 days after Friend virus inoculation. However, by 12 days after Friend virus inoculation, Sendai virus can once again induce amounts of interferon in the peritoneal cavity comparable with those induced in non-leukemic mice.

As seen in Fig. 2, Sendai virus induced increasingly larger amounts of interferon as detected in peritoneal washings when inoculated intraperitoneally at progressively longer intervals after Friend virus inoculation. However, Fig. 3 reveals that the higher titers of interferon produced in leukemic mice during the first 24 hours after injection of Sendai virus were not associated with significantly prolonged production of interferon as compared with control mice.

Effect of Statolon on survival of mice with established Friend virus leukemia. The demonstration that Sendai virus can both prolong survival in Friend virus-infected mice when inoculated 30 days after Friend virus and induce large amounts of interferon in leukemic mice suggested that interferon might be involved in the inhibition of Friend virus disease. Experiments were therefore conducted to determine if another inducer of interferon, i.e., Statolon, could also extend survival times of leukemic mice. It has been demonstrated (7) that a single injection of Statolon given between 6 days prior to and 3 days after Friend virus could completely protect over 50% of mice and markedly inhibit leukemia in the remainder.

Friend virus was inoculated into 90 mice on day 0, and 28 days later, sets of 30 mice each were injected with saline i.p., with Statolon (5 mg i.p.) or with Sendai virus ($10^{8.2}$ EID_{50} per mouse, i.p.). Subsequent deaths were recorded. As seen in Fig. 4, Statolon prolonged the life of mice with established Friend virus disease, and the prolongation of life was nearly identical to that produced by Sendai virus inoculated 28 days after FV.

Interferon production in leukemic and control mice in response to either Sendai virus or Statolon injections. Since survival of Friend virus-infected mice was prolonged to much the same degree by inoculation of either Sendai virus or Statolon, it seemed reasonable that interferon production might be involved in each case. A comparison was therefore made of the amount of interferon produced in leukemic mice in response to inoculation with Sendai virus or with Statolon.

As seen in Table I, more peritoneal interferon was detected in leukemic mice after inoculation with Sendai virus than with Statolon, whereas higher titers of serum interferon were attained in response to Statolon. Both Sendai virus and Statolon induced larger amounts of interferon when inoculated into leukemic mice 30 days after Friend virus than when inoculated into normal control mice; it should be noted also that, in contrast to leukemic mice, no peritoneal interferon was

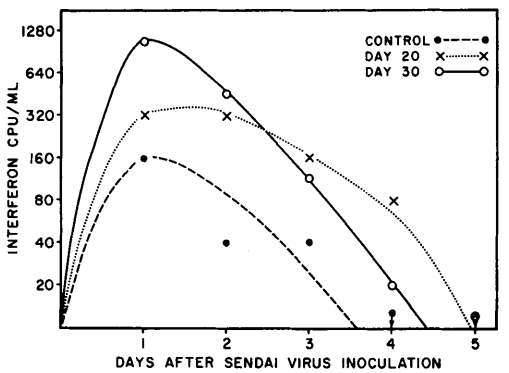

FIG. 3. Time course of interferon production in the peritoneal cavities of normal and leukemic mice inoculated with Sendai virus.

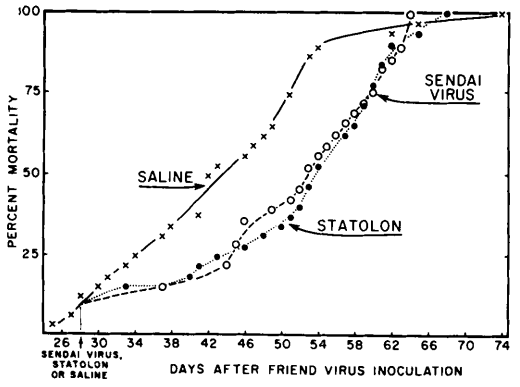

FIG. 4. Prolongation of life of mice inoculated intraperitoneally with Sendai virus or Statolon 28 days following Friend virus.

detected in control mice which received Statolon.

Augmentation of protective effects of Sendai virus by Statolon. The ability of Sendai virus or of Statolon to prolong the life of leukemic mice when inoculated 30 days after Friend virus suggested that these two agents might be effectively employed sequentially. Accordingly, 100 mice were inoculated with Friend virus, and 29 days later, 66 of the mice were inoculated with Sendai virus. At the end of the protective period induced by Sendai virus, i.e., when mice began to die at the same rate as the Friend virus control mice, half of the surviving animals in the Sendai virus group were injected with Statolon, 5 mg per mouse, i.p.

Figure 5 illustrates the results of this experiment and indicates that Statolon injected 24 days after Sendai virus can extend the life of leukemic mice beyond the usual period of prolonged survival induced by Sendai virus alone. It was also found that this injection of Statolon induced high titers of interferon in both the peritoneal exudates and sera of leukemic mice.

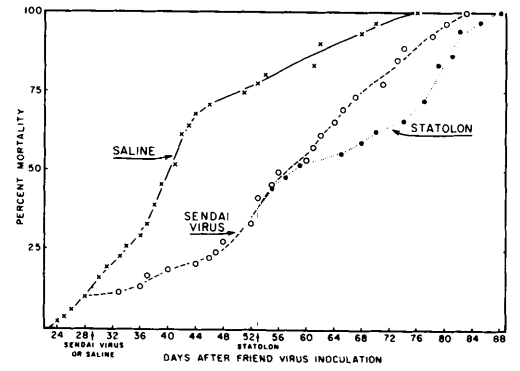

FIG. 5. Effect of sequential administration of Sendai virus and Statolon on survival of leukemic mice. Sendai virus was inoculated 29 days after Friend virus and Statolon 24 days after Sendai virus.

TABLE I. Interferon Production in Friend Virus-Infected Mice in Response to Inoculation with Sendai Virus or Statolon.

	Interferon titer in culture-protecting units[b]			
	Peritoneal wash[c]		Serum[c]	
Inoculum/ mouse[a]	Control	Friend virus-infected	Control	Friend virus-infected
Sendai virus $10^{8.2}$ EID_{50}	256	1024	192	384
Statolon 5 mg	<10	204	720	1280

[a] Sendai virus or Statolon was inoculated intraperitoneally into either normal mice or into mice inoculated with Friend virus 30 days previously.

[b] Culture-protecting units are calculated as the reciprocal of the highest dilution of the specimen, 1 ml of which significantly protected monolayers of mouse L cells against vesicular stomatitis virus.

[c] Each result represents the average of determinations in 5 mice. The peritoneum of each mouse was washed with 5 ml of saline. Peritoneal and serum samples were collected 24 hours after inoculation of either Sendai virus or Statolon.

Failure of sequential inoculations of Sendai virus to further prolong the life of leukemic mice. Sequential inoculations of Sendai virus in leukemic mice were given in an attempt to extend survival times beyond those which followed a single inoculation of Sendai virus. As noted in previous sections, inoculation of Sendai virus into mice 30 days after Friend virus prolonged survival. However, a second inoculation of Sendai virus 18 days after the first produced no additional prolongation of life. Furthermore, the second inoculation of Sendai virus failed to induce detectable amounts of interferon in the peritoneal exudates of leukemic mice.

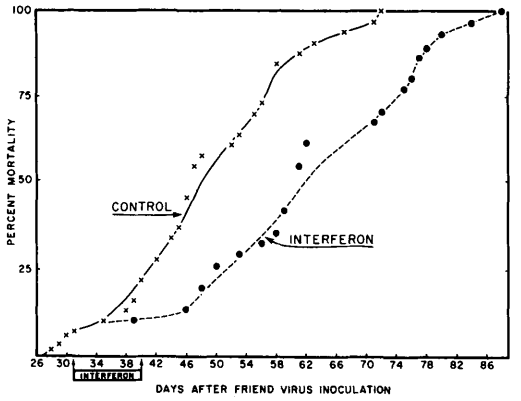

FIG. 6. Effect on survival of leukemic mice of peritoneal injections of interferon (10,240 CPU per mouse per day) beginning 31 days after Friend virus inoculation and continuing daily for 10 days. Control mice were injected at the same dosage schedule with a preparation containing no interferon.

Effect of injections of interferon on survival of leukemic mice. The demonstrations that Sendai virus and Statolon, both inducers of interferon production, could prolong life when inoculated into leukemic mice suggested that the mechanism responsible for the prolonged survival might involve interferon. Accordingly, interferon was prepared in mice inoculated with Newcastle disease virus as described in *Materials and Methods*, and a control preparation was obtained from mice not inoculated with NDV. The total protein content of the two preparations was equilibrated. Beginning 31 days after Friend virus inoculation, 30 mice received i.p. injections of the interferon preparation (10,240 CPU in a 0.2-ml volume per mouse) daily for 10 days, while 30 control mice received daily i.p. injections of the control preparation. The results of this experiment are illustrated in Fig. 6; leukemic mice injected with the interferon preparation survived for an average of 9 days longer than control mice.

The observation that interferon produced exogenously could prolong life when injected daily into leukemic mice for 10 days prompted us to extend the period of injections of interferon until all mice succumbed to leukemia. Beginning 31 days after Friend virus inoculation, groups of 30–40 mice were injected i.p. with 10,240 CPU of interferon daily until time of death. A control preparation containing no interferon was injected daily into an equal number of leukemic mice. Comparison of Figs. 6 and 7 indicates that daily administration of interferon beginning 31 days after FV inoculation and continuing until death of all mice resulted in survival times which did not differ appreciably from those of leukemic mice treated with interferon daily between the thirtieth and fortieth days only.

Discussion. The experiments presented herein represent an attempt to employ the principles of virus interference in the treatment of established virus-induced leukemia in mice. Three different interfering agents were selected for study: (*a*) Sendai virus, previously reported to inhibit Friend virus leukemia when inoculated prior to Friend virus (3); (*b*) Statolon, previously reported to inhibit Friend virus disease either partially or completely when inoculated between 6 days before and 7 days after Friend virus (7); and (*c*) interferon, previously reported to inhibit the splenomegalic response to Friend virus when repeatedly injected intraperitoneally for 1 month beginning either 1 day prior to or 2 days after Friend virus inoculation (8,9). In the present study it was found that Sendai virus, or Statolon, or repeated injections of

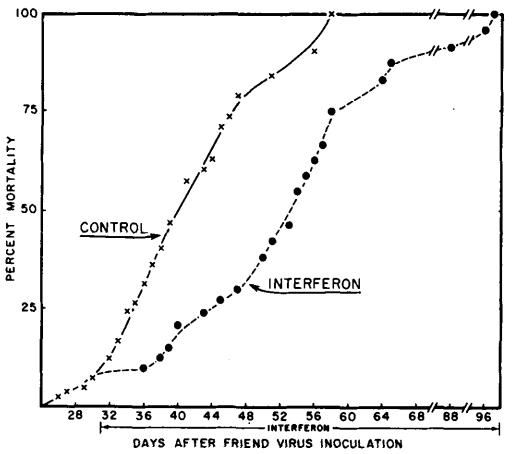

FIG. 7. Effect on survival of leukemic mice of peritoneal injections of interferon (10,240 CPU per mouse per day) beginning 31 days after Friend virus inoculation and continuing daily until death. Control mice were injected at the same dosage schedule with a preparation containing no interferon.

interferon, each prolonged the life of leukemic mice when administered 30 days after Friend virus inoculation, at a time when mice were otherwise beginning to die of leukemia. It is possible that both Sendai virus and Statolon prolonged life through the action of interferon since these two agents were shown to induce large amounts of interferon in leukemic mice and interferon itself was able to prolong survival.

The ameliorating effect of interferon on the course of established Friend virus leukemia represents a therapeutic application of this virus-inhibitory protein. Gresser *et al.* (9) have found that interferon preparations injected repeatedly into mice beginning 48 hours after Friend virus inoculation can reduce the splenomegalic response to Friend virus. However, no attempts were made to determine whether the Friend virus infection was in fact established at the time of the first interferon injection, and the existence of small spleens in some of the Friend virus control mice precludes a clear assessment of the therapeutic effectiveness of interferon in that study. Other investigators have found that interferon produced little or no suppression of established *in vivo* viral infections (10–15), although it is difficult to evaluate the therapeutic efficacy of interferon in these reports which dealt with viral diseases of different etiology and pathogenesis. Lampson *et al.* (13), in a study of the effect of interferon on an RNA tumor virus, found that treatment of chickens with purified interferon was without measurable success when it was applied as early as 6 hours after inoculation of Rous sarcoma virus. In addition they reported that interferon injected when pin-point size tumors first appeared did not cause regression or limit the eventual size of the tumor (13).

The mechanism by which injections of interferon itself prolonged the survival of mice with established Friend virus leukemia is problematic. Recent *in vitro* studies with nontumor viruses have revealed that interferon acts intracellularly at an early stage of virus infection (16–18). In accord with this concept is the observation that interferon has no effect on the neoplastic transformation of a cell by a DNA tumor virus when applied after the virus genome has become integrated into the cellular DNA (19,20). It has not yet been shown, however, that cells transformed by RNA tumor viruses, such as the murine leukemia virus group, are similarly resistant to the action of interferon. The possibility exists, therefore, that interferon may halt the expression of Friend virus within cells already infected by the virus, and thereby inhibit the malignant behavior of the cell with reduction in the mitotic rate. An alternative mode of action of interferon, and one which more closely conforms to present concepts, is that interferon injected into mice at an advanced stage of leukemia may protect normal cells from infection and neoplastic alteration by Friend virus. A requisite for this mechanism would be that susceptible host cells become infected by Friend virus throughout the course of the leukemic disease, a hypothesis not yet confirmed by experiment. A further requisite for this mechanism is that the accumulation of leukemic cells resulting from infection of normal cells late in the course of the disease leads to death of the animal. The consequence of either of the proposed mechanisms of interferon action would be to limit the increase in the numbers of leukemic cells.

Mice treated with interferon daily from the thirtieth day after inoculation of Friend virus until death did not live appreciably longer than leukemic mice which received only 10 daily inoculations of interferon beginning on the thirtieth day; this observation suggests that after a period of 40 days, mice inoculated with Friend virus entered a phase in which leukemia was no longer suppressed by the interferon preparation employed in this study. Several possibilities, currently being explored, could explain this apparent change in sensitivity to interferon: (*a*) Following inoculation of Friend virus, new populations of cells may become infected up to approximately the fortieth day, after which time the number of susceptible cells that could be protected by interferon is depleted; (*b*) repeated inoculation of interferon may lead to the development of antibody which neutralizes interferon administered subsequently, although Gresser *et al.* (21) detected "no significant anti-interferon-like activity" in sera of Swiss mice in-

jected i.p. with interferon 1 day before Friend virus inoculation then twice daily thereafter for 4 or 6 weeks; and (*c*) mice may die of Friend virus leukemia only when malignant transformation of host cells reaches some critical degree. A small but constant fraction of the leukemogenic expression of Friend virus may escape the suppressive effects of interferon even when interferon is administered under optimal conditions of time and dosage. Continued replication of Friend virus in the presence of daily injections of interferon could therefore result in accumulation of the unsuppressed leukemogenic factor in excess of threshold values and results in death of the host. Under these circumstances, it is to be expected that prolonged administration of interferon would eventually fail to inhibit progression of the leukemia. That leukemic mice are not completely refractory to the leukemia-inhibiting effects of interferon after the fortieth day following inoculation of Friend virus is suggested by the observation that Statolon administered on the fifty-third day of the disease (24 days after an initial dose of Sendai virus) induced large amounts of interferon, with subsequent further prolongation of life.

The ability of Sendai virus to inhibit Friend virus disease was enhanced when it was inoculated into mice at progressively longer intervals after Friend virus inoculation. A distinction should be made between the possibility of increased sensitivity of mice to the leukemia-inhibiting effect of Sendai virus and the possibility of increased susceptibility to infection with Sendai virus during the course of Friend virus disease. One manifestation of increased susceptibility to Sendai virus infection in leukemic mice may be the enhanced production of interferon, perhaps reflecting the infection of more host cells by Sendai virus or more interferon production per infected cell. Studies of Sendai virus replication and the associated cytopathic effects in mice at various stages of leukemia should provide an explanation for these observations. At present, one cannot exclude the possibility that mice in advanced stages of virus-induced leukemia become increasingly sensitive to the interferon induced by Sendai virus or to other leukemia-inhibiting mechanisms invoked by Sendai virus, such as oncolysis.

The available data indicate that a quantitative relationship exists between the amount of interferon induced by Sendai virus and the extent of inhibition of Friend virus leukemia. Notably, interferon induced by Rauscher leukemia virus (RLV) has been implicated in the resistance of adult CD-1 mice to RLV (22), and further, susceptible suckling (22) but not adult (23) mice can be rendered resistant to RLV by interferon. In a previous study it was shown that injections of Sendai virus between 1 and 10 days after Friend virus induced considerably less interferon in leukemic mice than in controls and failed to inhibit the splenomegalic response to Friend virus (2). In the present study, Sendai virus inoculated into leukemic mice 20 days after Friend virus failed to prolong life appreciably, whereas administration of Sendai virus 30 days after Friend virus prolonged life significantly. Large amounts of interferon were produced in response to Sendai virus inoculated at each time, although more interferon was produced at 30 days than at 20 days. It is possible that the higher titers of interferon produced at 30 days exceed threshold levels of interferon required to inhibit Friend virus leukemia significantly. Alternatively, as proposed above, at 30 days after Friend virus inoculation mice may be more sensitive to the leukemia-inhibiting effects of interferon than at 20 days. A final correlation between interferon production and leukemia inhibition is the observation that Sendai virus administered a second time approximately 2 weeks after its initial inoculation 30 days after Friend virus could neither induce interferon nor further prolong the life of leukemic mice. The absence of interferon production in response to the second inoculation of Sendai virus may have been due to depletion of host cells susceptible to Sendai virus infection or to neutralization of the second dose of Sendai virus by homologous antibodies in peritoneal exudates.

The formation of antibodies to a nontumor virus employed in the treatment of leukemia may necessitate the sequential administration of a variety of antigenically different viruses

as was done in a clinical study previously reported (1). An alternative approach is provided by the ability of Statolon to prolong survival of leukemic mice when administered approximately 3 weeks after Sendai virus. These observations suggest that a variety of agents may be combined with nontumor viruses in effective treatment of mice with established viral leukemia.

The application to human leukemias of results obtained from interference studies with virus-induced murine leukemia systems remains speculative. However, the prolongation of life which followed inoculation of a nontumor virus into mice with established virus-induced leukemia may correspond to the effects observed in a previously reported study of a patient with acute myelogenous leukemia (1). In that study, intravenous inoculations of large doses of viruses induced interferon and were followed consistently by transient clinical and hematologic remissions which led to a prolongation of the patient's life.

Summary. DBA/2 mice are 100% susceptible to Friend leukemia virus and begin to die 26 days after virus inoculation. Sendai virus (parainfluenza 1) inoculated intraperitoneally into leukemic mice 30 days after Friend virus prolonged survival by 12 days. Evidence that Sendai virus prolongs the life of leukemic mice through the action of interferon derives from the following observations: (*a*) Sendai virus induced large amounts of interferon; (*b*) Statolon (an extract from the mold *Penicillium stoloniferum*), when injected 28 days after Friend virus, also induced large amounts of interferon and prolonged survival by 11 days; and (*c*) interferon itself injected intraperitoneally daily for 10 days commencing 31 days after Friend virus prolonged survival by an average of 9 days. However, when interferon injections were continued daily until death of the mice, survival was no longer than in mice which received only 10 daily injections. This study represents a successful application of interferon as a therapeutic agent in an established virus-induced neoplastic disease. However, the development in mice of a state refractory to the leukemia-inhibiting effects of interferon after 10 days' administration indicates that interferon may not be effective in the long-term treatment of virus-induced leukemia.

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