Inhibition of Friend Virus-Induced Splenomegaly by an Associated Lymphatic Leukemia Virus[1] (34332)

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A virus that induces lymphatic leukemia (LLV) can readily be obtained from preparations of Friend virus (FV) by inoculation into rats (1, 2). While LLV can be freed from FV, numerous attempts to obtain preparations of FV that will not induce lymphatic leukemia have been unsuccessful. This suggests that a special relationship may exist between the two viruses and that LLV may be necessary for the replication of FV. Antigenic studies have failed to reveal differences between preparations of Friend virus (containing LLV) and LLV freed from FV.

Rowe (3) and Dmochowski *et al.* (4) showed that Moloney and Gross viruses, respectively, will interfere with the splenomegalic response to FV. Since our strain of FV could not be separated from LLV it seemed important to determine if prior infection with this particular lymphatic leukemia-inducing virus would affect the course of Friend disease (FD).

Materials and Methods. Young adult female BALB/c mice were obtained from either Simonsen Laboratories, Gilroy, California, or Flow Laboratories, Rockville, Maryland. The origin of our strains of FV and LLV has been reported (5, 6). The FV pools came from passages 10 and 16 in BALB/c mice; the latter had been through 2 terminal dilution procedures in an unsuccessful effort to remove the associated LLV (1). The LLV which has been shown to be free from FV (1) came from the eighth passage in Scott-Russ rats.

The standard methods used for the preparation of virus pools, and the titration of virus and antibody have been described (5, 6). Titrations were performed in young BALB/c mice; these were killed 35 days after inoculation in virus assays and 45 days after inoculation in antibody titrations. Suspensions of normal Scott-Russ rat or BALB/c mouse spleen, liver, and thymus were prepared in the same manner as the virus pools.

6-Mercaptopurine (6MP), obtained from Dr. A. P. Kimball, was dissolved in saline, and inoculated ip. Dosage, which had previously been shown to be nontoxic, was 30 mg/kg of body weight/day for 8 days.

Results. In 2 initial experiments groups of BALB/c mice were inoculated ip either with $10^{3.5}$ ID_{50} of LLV or an equal volume of a comparable suspension of normal rat tissue. At intervals from 1 to 21 days later mice were challenged ip with approximately $10^{3.1}$ ID_{50} of FV. Additional groups received either LLV or FV. Twenty-one days after challenge mice were killed and their spleens were weighed; those weighing less than 500 mg were examined histologically. The results of both experiments are combined in Table I.

Inoculation of LLV 21 days before challenge with FV resulted in a striking reduction in the incidence of FD from 100% among control mice to 15% (4 of 26) among test mice. The extent of disease judged both by spleen size and histologic appearances was also very much less than that seen among control mice. When the interval between inoculation with LLV and challenge with FV was cut to 14 or 7 days there was a moderate inhibition of splenic enlargement and reduction in the incidence of FD to 15/20 (75%) and 13/17 (76%), respectively. When the

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TABLE I. Inhibition of Friend Disease by Previous Inoculation with Lymphatic Leukemia Virus.

Initial inoculum	Time interval (days)	Challenge	Results	
			Mean spleen wt (mg)	No. with FD/ No. inoculated[a]
LLV	21	FV	146 ± 100[b]	4/26
NR[c]	21	FV	1380 ± 590	30/30
LLV	14	FV	680 ± 430	15/20
NR	14	FV	1240 ± 443	20/20
LLV	7	FV	550 ± 547	13/17
NR	7	FV	1050 ± 269	20/20
LLV	3	FV	1270 ± 467	20/20
NR	3	FV	1300 ± 528	19/20
LLV	1	FV	1830 ± 302	10/10
NR	1	FV	1980 ± 412	10/10
LLV	21	None	118 ± 37	0/20
None	21	FV	1680 ± 448	20/20

[a] Determined on a basis of spleen size and histologic examination.
[b] ± standard deviation.
[c] NR = suspension of normal rat spleen, liver, and thymus.

interval between inoculation of the two viruses was reduced to 3 days or less, there was reduction neither in the incidence of Friend disease nor spleen size. It was concluded that previous inoculation with LLV inhibited the development of FD, particularly if the time interval between the two viruses was 21 days.

These experiments did not provide information about the subsequent fate of test mice that apparently did not have Friend disease at the time the experiment was terminated, nor did they indicate the effect of LLV on FV replication. Since FV and LLV are related antigenically it was possible that the initial inoculum was inducing the formation of antibodies which neutralized FV. Therefore, it was decided to measure the antibody response 21 days after inoculation with LLV and treat one group of mice which received LLV with 6MP. The latter is known to depress the immune response of mice (7) and does not significantly affect FV replication (8).

Groups of BALB/c mice were inoculated ip with either $10^{3.5}$ ID_{50} of LLV or an equivalent amount of normal rat tissue suspension. Part of each group was then inoculated ip with 30 mg/kg of body weight of 6 MP on 8 consecutive days. Twenty-one days after inoculation of virus, part of each group was killed, their serum was collected, and tested for the presence of neutralizing antibodies to FV. The remainder were challenged with $10^{3.3}$ ID_{50} of FV or an equivalent suspension of normal mouse tissue. They were killed 21 days later and their spleens were weighed. One-half of each spleen was taken aseptically for bioassay, the other was examined histologically. Two further groups of mice were inoculated with LLV; one was challenged with FV 21 days later and the other with a suspension of normal mouse tissue. Both groups were kept until all the mice were dead or moribund.

The results are given in Table II. Again there was a significant reduction in the incidence of FD among mice preinoculated with LLV compared with those receiving normal rat tissue ($p < 0.05$), as well as marked inhibition of splenomegaly. Histologically, FD in the test mice was limited to only a few small foci of proliferating stem cells. When inoculation of LLV was followed by treatment with 6MP there was a slight fall in the incidence of FD and some inhibition of splenomegaly. No neutralizing antibody to FV was detected

TABLE II. Effect of Previous Inoculation of Lymphatic Leukemia Virus with and without Subsequent Treatment with 6-Mercaptopurine on Friend Virus Replication and Friend Disease.

Initial inoculum	Antibody titer at 21 days[a]	Challenge	Results: Mean spleen wt (mg)	Results: No. with FD/ No. inoculated	Results: FV titer[b]
LLV	<1:4	FV	260 ± 32[c]	5/10	3.9
		NM[d]	110 ± 16	0/10	—
LLV[e]	<1:4	FV	690 ± 427	8/10	4.5[f] 3.0[g]
		NM	140 ± 21	0/10	—
NR	<1:4	FV	1890 ± 367	10/10	4.5
NR[e]	<1:4	FV	1090 ± 491	9/10	3.9

[a] Fifty percent neutralizing endpoint against 20 ID_{50} of FV.
[b] ($-\log_{10}$ ID_{50} per ml).
[c] ± standard deviation.
[d] NM = normal mouse spleen, liver, and thymus.
[e] Followed by treatment with 6MP.
[f] Spleens weighing greater than 500 mg.
[g] Slpeens weighing less than 500 mg.

21 days after inoculation with LLV. Bioassay of FV in the spleens of mice at the end of the experiment revealed no significant differences in the amount of virus present in the various groups. So that while prior inoculation with LLV inhibited the development of FV-induced splenomegaly it had not interfered with FV replication. This was born out by the fact that 16 of 20 mice challenged with FV 21 days after inoculation with LLV and kept until dead or moribund, died with advanced FD from 47 to 134 days after inoculation. One additional mouse showed FD in the spleen and lymphatic leukemia in the thymus. Nine of 10 mice inoculated with LLV and challenged with normal mouse tissue died with lymphatic leukemia.

Discussion. The lymphatic leukemia-inducing virus used in these studies was originally isolated from rats inoculated with FV. Evidence to date indicates that it is present in all preparations of the latter. It has been postulated that this is not fortuitous, but that LLV may act as a helper virus for FV (1). If this were true, then it might be wondered why the addition of LLV to FV does not cause enhancement. However, it is known that all our preparations of FV contain LLV and that the addition of further LLV does not increase the yield of FV (unpublished observations).

A number of possible mechanisms can be postulated to explain the repression of FD. That it is interferon mediated seems unlikely since FV infection has been reported not to result in interferon production (9). Further, there was no difference in the virus titer between test and control mice, nor were there data to suggest that neutralizing antibodies were formed following inoculation with LLV which might cross react with FV. Rich and colleagues (10) in a preliminary communication report that prior inoculation with Rich leukemia virus resulted in a decreased sensitivity to the development of FD. Neutralizing antibodies against FV were not demonstrated in their experiments.

The observation that previous inoculation with LLV induced resistance to subsequent challenge with cells of a virus-free Friend virus-induced tumor (unpublished data) suggests that the homograft rejection response might be involved. It might be postulated that the host forms antibodies to new transplantation antigens induced by LLV which cross react with FV-transformed reticulum cells. Presumably as the FV titer rises the number of cells transformed increases to a

point where this defense mechanism is overwhelmed. Finally, it is possible that both viruses affect the same hematopoietic stem cell and that the inhibiton of FV-induced splenomegaly is due to the infection initially of the majority of these cells by LLV. A choice between these hypotheses cannot be made in the absence of additional data.

Summary. Friend virus was shown to be invariably associated with a virus that induces lymphatic leukemia. Prior inoculation with the latter inhibited Friend virus-induced reticulum cell proliferation in the spleen, but did not interfere with Friend virus replication. This effect was most marked when the interval between inoculation of the 2 viruses was 21 days.

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