CONTINUOUS REPLICATION OF FRIEND VIRUS COMPLEX
(SPLEEN FOCUS-FORMING VIRUS-
LYMPHATIC LEUKEMIA-INDUCING VIRUS)
IN MOUSE EMBRYO FIBROBLASTS

Retention of Leukemogenicity and Loss of Immunosuppressive Properties*

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The Friend virus (FV)[1] and Rauscher leukemia virus (RV) complexes are unique among the murine leukemia viruses (MuLV) in that they contain a rapidly acting, erythroid leukemia-inducing, spleen focus-forming virus (SFFV). Both the Friend and Rauscher strains of SFFV are defective for spleen focus formation in vivo, are leukemogenic in susceptible adult mice, and can therefore be easily detected and quantified in vivo (1,2). However, early efforts to develop an in vitro assay for SFFV and to maintain this virus in vitro have not been successful (3). To date, the only in vitro source of Friend SFFV has been FV-induced erythroleukemic spleen cells maintained in suspension culture (4–6). Virus obtained from these suspension cultures can be readily detected in susceptible adult mice via the induction of spleen foci and erythroleukemia. More recently, SFFV function has been successfully detected in vitro using a virus interference test in conjunction with the XC cell plaque assay (7).

Since it is now possible to detect SFFV function in vitro [i.e., in mouse embryo fibroblast (MEF) cultures], it was of interest to determine if SFFV could infect and replicate in MEF cultures, and to what extent in vitro-grown SFFV maintained or lost those biological properties normally ascribed to in vivo-maintained FV complex (i.e., SFFV-LLV). In the FV and other leukemia virus systems, it has been noted that extended in vitro growth in MEF is associated with "attenuation" of viral oncogenic potential in vivo (8–13). Only a recent study utilizing the Gross leukemia virus (GLV) suggests that fully leukemogenic MuLV can be derived from monolayer cultures (14). However, the source of cells for these experiments was hyperplastic thymus tissue obtained from 8-wk old GLV-infected C_3H mice. In contrast, I report in the present study

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[1] Abbreviations used in this paper: BI, blastogenic index; Con A, concanavalin A; FFU, focus-forming units; FV complex, Friend virus complex; FV-TC, tissue culture (i.e. in vitro-derived) FV complex; GLV, Gross leukemia virus; LLV-F, Friend lymphatic leukemia-inducing virus; MEF, mouse embryo fibroblasts; MolLV, Moloney leukemia virus; MSV, murine sarcoma virus; MuLV, murine leukemia virus; PFU, plaque-forming units; RadLV, radiation leukemia virus; RV complex, Rauscher virus complex; SFFV, spleen focus-forming virus; Swiss-FV cells, NIH Swiss embryo fibroblasts infected with FV complex.

that both defective Friend SFFV and its associated LLV-F helper can productively infect NIH Swiss MEF, and that virus stocks (SFFV and LLV-F) derived from this cell culture system are fully leukemogenic in mice for the induction of erythroleukemia and lymphatic leukemia, respectively. SFFV contained in the in vitro-derived FV complex (here referred to as FV-TC) has an NB-tropic host range (15) for the induction of foci in vivo. In addition, FV-TC does not immunosuppress the responsiveness of murine lymphocytes to concanavalin A (Con A) in vitro. Rather, FV-TC acts as a mitogen to nonspecifically stimulate the proliferation of BALB/c thymocytes.

Materials and Methods

Mice. Male and female 4- to 6-wk old BALB/c, C3H/He, C57BL/6Ha, C58 waved, DBA/2Ha, and 129 mice were obtained from our own breeding colony. In addition, ABT/Ha mice were originally obtained through the courtesy of Dr. T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y. and are now maintained in our breeding colony. These highly inbred mice (>50 generations) are shown to be homozygous belted (*bt*) (16), *H-2^d*, and *Fv-2^{rr}* (17).

Viruses. SFFV, as contained in the NB-tropic (15) strain of FV complex, had undergone 14 cell-free passages in adult BALB/c mice when it was used in an attempt to infect NIH Swiss MEF. The isolation and maintenance of the LLV-F stocks used has been previously described (18).

Virus Titration. All SFFV preparations were titrated in fully susceptible adult mice with the spleen focus assay method (19). Virus titers were expressed in focus-forming units (FFU) per milliliter. In addition, all preparations of FV complex were titrated in BALB/c MEF using the XC plaque assay (20). Plaques were counted macroscopically and also viewed microscopically for the presence of syncytial giant cells. It was possible to obtain titer estimates in plaque-forming units (PFU) per milliliter for LLV-F contained in the FV complex with SFFV. LLV-F is always present in the FV complex 10- to 100-fold excess over SFFV. LLV can therefore be isolated free of SFFV via endpoint dilution techniques. We can therefore simultaneously determine the dose of both SFFV (in FFU) and LLV-F (in PFU) given to a susceptible mouse or used as an inoculum for an XC plaque assay.

Microplate Culture System for T-Cell Mitogens. FV preparations were tested for their ability to either suppress the proliferative response of normal mouse lymphocytes to Con A in vitro or to independently stimulate mouse thymocytes (i.e., act as a nonspecific mitogen for T cells) as follows: nucleated BALB/c thymus cells were cultured in Falcon Microtest II plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) at 37°C for 3 days with either 1 μg of Con A, FV, or both. Control cultures received neither the T-cell mitogen nor FV. 0.5 μCi of [^{3}H]thymidine was used to label proliferating cells synthesizing DNA, and the cell suspensions were harvested with a Microbiological Associates MASH II automatic harvester (Bethesda, Md.). The radioactivity in filter discs was measured in a liquid scintillation counter. The mitogenic response of the cultured T cells is presented in terms of the blastogenic index (BI). The BI is defined as the ratio of the result of the test culture to the corresponding control for mitogen response (cpm [^{3}H]thymidine with mitogen/cpm no mitogen).

Results

Productive Infection of Swiss MEF by Friend SFFV. It has been established that Friend LLV, like other MuLV's, can infect MEF and induce XC plaques (7). However, it has not been established if Friend SFFV can productively infect MEF. The only hint that defective SFFV is infectious for MEF comes from the observation that defective SFFV can "function" in a fibroblast test system to directly interfere with MuLV-induced XC plaque formation in NIH Swiss and BALB/c cells. SFFV interference is a heat-labile property directly associated with the defective virus particles (7). After this apparent detection of SFFV function and possible infectivity for MEF, I set out to determine if SFFV could productively infect NIH Swiss cells. Approximately 2×10^5 Swiss cells contained

in a 75-cm^2 plastic T-flask were DEAE-dextran-treated for 1 h. This culture was next exposed to 5×10^4 FFU of Friend SFFV as contained in 5 ml of fresh medium. This preparation of FV complex is NB-tropic and the inoculum contained approximately 8.4×10^5 PFU of NB-tropic LLV-F. After an adsorption period of 1 h, an additional 10 ml of fresh medium was added to the flask. Media was subsequently changed every 24 h for 5 days and assayed for the presence of infectious SFFV as described in Materials and Methods. A small quantity of detectable infectious SFFV remained in the media sample on day 1, and may represent unadsorbed SFFV from the original inoculum. After 7 days of culture, the Swiss cells exposed to FV complex (Swiss-FV cells) were trypsinized and 10^5 cells were passaged into a new T-flask. 24 h before this and each subsequent passage, the cells were rinsed with phosphate-buffered saline (PBS) and 10 ml of fresh media was added. All such samples were assayed for the presence of infectious SFFV released into the media over the 24-h period. It is possible to express the amount of infectious SFFV present in these 10-ml media samples as SFFV recovered per 10^6 Swiss-FV cells since the cells were removed from the T-flask by trypsinization and counted using a Coulter cell counter at the time of media collection. The results presented in Fig. 1 show that ≤ 100 FFU of detectable SFFV were recovered per 10^6 Swiss-FV cells between days 7–20, while approximately 10^4 FFU of SFFV were recovered per 10^6 cells on day 28 with maximum titers recovered after 50 days. This time represents the 7th subculture of Swiss-FV cells. The total amount of infectious SFFV recovered on and after day 28 exceeds the titer of the original SFFV inoculum by sixfold, indicating that Friend SFFV is truly replicating. Swiss-FV cells have now been passaged at weekly intervals for 25 mo and continue to produce $\geq 10^4$ FFU of SFFV per 10^6 cells.

Electron microscopic observations of Swiss-FV cells prepared from concentrated culture media have revealed numerous type-C virus particles in cytoplasmic vesicles, budding from the cytoplasmic membrane, and free in the extracellular space. No evidence of mycoplasma growth was present in control or infected culture media.

Immunostimulatory (Mitogenic) Properties of In Vitro-Derived FV complex. The proliferative responses that occur in vitro after exposure of murine lymphocytes to nonspecific agents such as phytohemagglutinin (PHA) and concanavalin A (Con A) have been shown to be directly suppressed in vitro by RV complex (21), and FV complex (Kumar and Eckner, unpublished observations), both of which contain SFFV and LLV, and by the Moloney (MolLV) and Gross (GLV) strains of MuLV (22). To determine if in vitro-derived FV complex (i.e., FV-TC preparations) could also suppress the proliferative response of murine T cells to Con A, BALB/c thymocytes were cultured in the presence or absence of T-cell mitogen with and without various dilutions (80–100 FFU) of several FV-TC preparations. All FV-TC preparations tested failed to suppress the response of BALB/c thymus cells to Con A. FV harvested at the 6th and 9th subcultures of Swiss-FV cells (FV-TC6 and FV-TC9, respectively) neither suppressed nor stimulated. However, FV complex derived from the 12th (FV-TC12) and subsequent subcultures was mitogenic for BALB/c thymus cells in vitro (Fig. 1). To further investigate the mitogenic nature of FV-TC preparations,
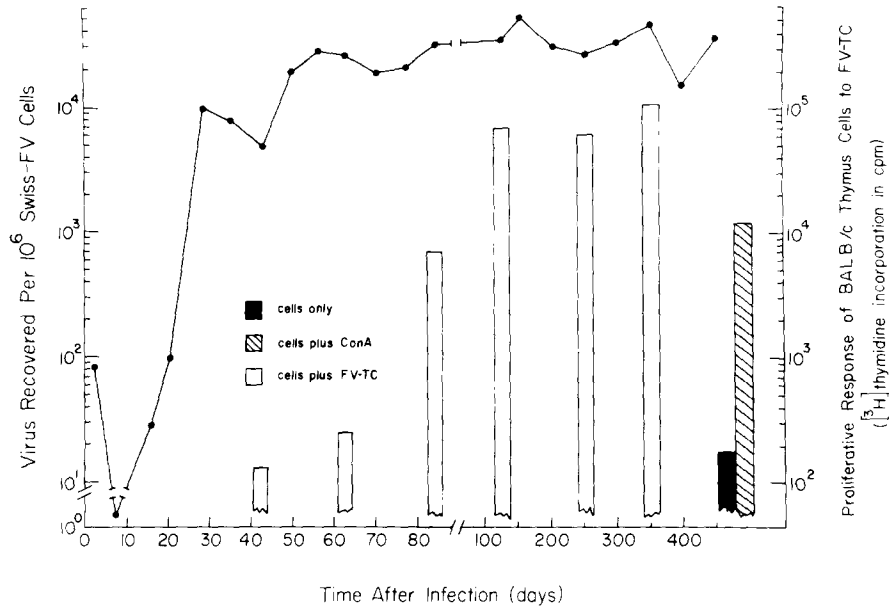
Fig. 1. Replication of Friend SFFV in NIH Swiss mouse embryo fibroblasts. The bar graph beneath the corresponding in vitro-derived SFFV titer estimates in FFU summarizes the results of experiments designed to determine the ability of several different FV-TC preparations to act as a nonspecific mitogen for BALB/c thymus cells.

virus from the 18th subculture of Swiss-FV cells (FV-TC18) was prepared and assayed for the presence of both SFFV and LLV-F. As shown in Table I, the FV-TC18 preparation was mitogenic for BALB/c thymus cells at all virus doses used ranging from 80 to ≤1.0 FFU of SFFV per microtest plate well. This result, as well as the results of experiments using LLV isolated from FV-TC stock, indicate that in vitro-derived LLV-F is of itself fully mitogenic at doses as low as 10 PFU per microtest plate well (Table I, Exp. 1). Further, the mitogenicity of LLV without SFFV present is virtually identical to that observed when SFFV was present, suggesting that SFFV does not directly contribute to this response.

When both FV-TC18 and Con A were added to the cultures, two different responses were observed which were dependent upon the virus dose. The highest FV-TC dose (80 FFU/10,000 PFU) diminished the Con A response by approximately 3,500 cpm. This may be due to mitogen overload in the test system since all other FV-TC18 doses used with Con A resulted in [^{3}H]thymidine incorporation values in cpm which were approximately additive when compared to the FV-TC18 and Con A controls (Table I, Exp. 1). The mitogenicity of in vitro-derived FV complex is both a heat-labile and UV-sensitive property of the virus (Table I, Exp. 2), as is the immunosuppressive property of in vivo-derived LLV-F. Further, FV mitogenic properties are directly associated with the virus particles since the immunostimulatory activity bands in sucrose and is present only if XC plaque-forming activity is also present. Finally, supernatant fluids from uninfected NIH Swiss MEF cultures are not mitogenic in this test system (Table I, Exp. 2).

TABLE I

Effect of In Vitro-Derived FV Complex and LLV Only on the Proliferative Response of BALB/c Thymus Cells

FV preparation	Virus dose* SFFV (FFU/well) LLV (PFU/well)	[³H]Thymidine incorporation (cpm)‡		
		Control (BI)§	+ FV (BI)	+ FV and Con A (BI)
Exp 1				
None; cells only	—	225	—	—
None; cells + Con A	—	10,780 (48)	—	—
Cells + in vitro-grown SFFV-LLV (FV-TC18)	80/10⁴	—	23,370 (104)	7,310 (32)
	8/10³	—	11,300 (50)	17,500 (78)
	0.8/10²	—	7,580 (34)	14,700 (65)
	0.08/10	—	8,940 (40)	16,100 (72)
Cells + in vitro-grown LLV	—/10³	—	12,500 (56)	
	—/10²	—	7,900 (35)	
	—/10	—	7,050 (31)	
Exp. 2				
None; cells only	—	154	—	
None; cells + Con A	—	13,890 (90)	—	
Cells + FV-TC18	80/10⁴		18,720 (121)	
	8/10³		8,500 (55)	
	0.8/10²		5,740 (37)	
Heat-inactivated FV-TC18 (56°C for 60 min)	80/10⁴		230 (1.5)	
UV-inactivated FV-TC18	80/10⁴		276 (1.8)	
Sucrose gradient-purified FV-TC18				
Sample pool (1.12–1.14 g/ml)	<10/<10		500 (3.2)	
(1.15–1.17 g/ml)	10/500		8,060 (52)	
(1.18–1.20 g/ml)	<<10/≤10		200 (1.3)	
Resuspended FV-TC18 pellet	10/1,200		9,060 (59)	
"Normal" NIH Swiss culture fluid	—	200 (1.3)		
Exp. 3				
None; cells only	—	414		
None; cells + Con A	—	13,085 (32)		
Cells + FV-TC passaged once in vivo ‖	80/10⁴		259 (0.62)	339 (1.55)

* Virus titer estimates for SFFV were determined using the spleen focus assay in permissive BALB/c mice, while LLV titer estimates were determined in vitro (BALB/c MEF) using the XC plaque assay as described (20).

‡ After an incubation period of 48 h, each culture (i.e., each microtest plate well) received 0.5 μCi of [³H]thymidine. After 24 h of further incubation (optimum response), the cultures were harvested as described in Materials and Methods. The incorporation of [³H]thymidine was measured in a liquid scintillation counter and the blastogenic (proliferative) response of murine thymocytes was expressed in cpm.
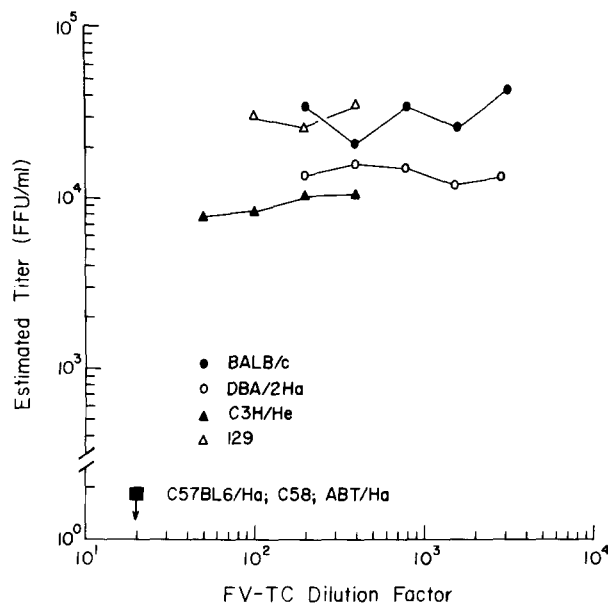
Fig. 2.   Dose-response relationships of in vitro-derived Friend spleen focus-forming virus (FV-TC18), as titrated in N-(Fv-1^{nn}) and B- (Fv-1^{bb}) type mice.

One in vivo passage of FV-TC18 resulted in the total loss of the mitogenic property with the reappearance of immunosuppressive properties (Table I, Exp. 3). This result demonstrates a clear association between in vivo growth of FV and its ability to suppress mouse thymocytes, and suggests that FV complex (SFFV-LLV) is not inherently immunosuppressive. While the mechanism of this interconversion between immunostimulatory and suppressive virus is unknown, both virus markers appear to be dependent upon the presence of infectious FV.

Host Range and Leukemogenicity (Focus Formation) of In Vitro-Derived SFFV.   The NB-tropic preparation of FV complex used to infect the NIH Swiss cells (as described above) contained fully leukemogenic SFFV and LLV-F. To determine if the in vitro-derived FV complex (specifically the FV-TC18 preparation referred to here as FV-TC) expresses the same host range in vivo for focus formation as the original preparation of FV complex used as source material, several different strains of N-type (Fv-1^{nn}) and B-type (Fv-1^{bb}) mice were inoculated with serial dilutions of this high titer FV-TC preparation obtained as an unconcentrated 24-h media harvest from the 18th subculture of Swiss-FV cells. As shown in Fig. 2, this FV-TC preparation effectively induced spleen foci in

§ Control cultures received either cells alone, or cells plus Con A only. The doses of Con A used in these experiments did not agglutinate FV since there was no reduction in the titer estimate of FV suspensions exposed to Con A. The blastogenic index (BI) is defined as a ratio of the result of the test culture with Con A, FV-TC, or both Con A and FV-TC added, to the corresponding control (cells only) for mitogen response.

|| Stock FV-TC18 (an in vitro-derived SFFV-LLV complex) was diluted 1:10 and 0.5 ml was injected intravenously in a group of six 4-wk old BALB/c mice. 9 days later, the enlarged erythroleukemic spleens were removed. A 20% (wt/vol) cell-free extract of leukemic tissue was prepared and stored at −196°C.

both N- and B-type mice. Only the SFFV-resistant Fv-2^{rr} (17) mice C57BL/6, C58, and ABT/Ha (16) were totally resistant to SFFV contained in FV-TC indicating that this virus is not able to overcome Fv-2 genetic resistance. From this data it can be concluded that FV-TC is NB-tropic for in vivo focus formation. Since SFFV-induced focus formation is "helper controlled", that is, directly dependent upon the ability of associated MuLV to provide a helper function for late stages in SFFV synthesis (1), it can be assumed that a high titer helper virus (presumably LLV) is present and functioning in FV-TC preparations.

Leukemogenicity of In Vitro-Derived Friend LLV. A high titer XC plaque-forming virus is present in FV-TC stocks in excess over SFFV ($\geq 10^6$ PFU/ml as detected in BALB/c cells), and can be isolated free of detectable SFFV via endpoint dilution techniques. To determine if this virus was leukemogenic when administered to susceptible newborn BALB/c mice, an experimental protocol was designed as follows: inocula of the same FV-TC preparation used in the host range study described above were prepared as serial 10-fold dilutions ranging from 1:100 to 1:10⁶. Each newborn BALB/c mouse received 0.1 ml of a given virus dilution intraperitoneally. All mice were subsequently observed for onset of splenomegaly and leukemia. As a control, a preparation of in vivo-derived NB-tropic FV complex was similarly diluted and inoculated intraperitoneally into groups of newborn BALB/c mice. As shown in Table II, the infectious SFFV dose contained in the FV-TC inocula ranged from 40 FFU to ≪1.0 FFU, while at each virus dilution, mice received a substantially higher infectious dose of LLV, the presumed plaque-forming virus present in all FV-TC preparations together with SFFV and present beyond the SFFV endpoint. This same relationship holds true for the in vivo-derived SFFV-LLV preparation. All LLV(PFU)/SFFV(FFU) ratios were determined utilizing a dose-response technique (7). Soon after the initiation of this experiment, mice given the highest SFFV doses (as contained in FV-TC preparations and in vivo-grown FV complex) began to die of erythroid leukemia (typical Friend disease). Further, infectious SFFV could be readily obtained from the spleen extracts of all such animals. After an average latent period of 70 days, all mice which had received approximately 1.0 FFU of SFFV (0.1–1.0 FFU) were dead and were shown to have had erythroid leukemia, polycythemia, and SFFV viremia (Table II). In contrast, most mice receiving <0.1 FFU of either in vitro- or in vivo-derived SFFV eventually succumbed to a lymphoid disease, with an associated anemia and high peripheral white blood cell counts. The liver sinusoids were heavily infiltrated with leukemic cells (Fig. 3 a). The lymph nodes and bone marrow contained leukemic infiltrates or were completely replaced by leukemic cells. However, the thymus was not involved. The spleens revealed either extensive myeloid metaplasia or complete replacement of normal hematopoietic elements with leukemic cells (Fig. 3 b). The cytological appearance of the leukemic cells varied between very poorly differentiated lymphoblasts to well-differentiated lymphocytes and the peripheral white blood cell counts varied between 80,000 and 100,000 cells/mm³ in leukemic mice. This is typical of Friend LLV-induced leukemia. In addition, no detectable SFFV was recovered from any of these animals while >10⁴ PFU of MuLV (presumably LLV-F) was easily detected. In experiments not presented here, it has been shown that the XC plaque-forming virus recovered from mice origi-

TABLE II

Pathogenicity of In Vitro- and In Vivo-Derived FV Complex in Newborn BALB/c Mice

Inoculum	Virus dose* SFFV (FFU/animal) LLV (PFU/animal)	Histopathology of leukemia induced Type‡	Avg. latent period	Frequency (no. leukemic/ no. inoculated)	Virus recovery§ SFFV	LLV
			days		*FFU/ml*	*PFU/ml*
In Vitro-grown SFFV-LLV	40.00/2,000	Ery	12	7/7	>10³	NT
	4.00/200	Ery	27	7/7	>10³	NT
	0.40/20	Ery	70	7/7	4 × 10³	NT
	0.04/2	Lym	86	3/5	0	2.6 × 10⁵
	0.004/0.2	Lym	102	2/6	0	8.0 × 10⁵
In Vivo-grown SFFV-LLV	5.00/300	Ery	47	7/7	>10³	NT
	0.50/30	Ery	60	7/7	3 × 10³	NT
	0.05/3	Lym	194	4/6	0	10⁵
	0.005/0.03	Lym	124	3/7	0	9 × 10⁴
None	0/0	None	—	0/5	0	0

NT = not tested. Since SFFV is known to interfere with LLV plaque formation in the XC test (Eckner et al., 1975), all leukemic spleen extracts known to contain SFFV were not assayed for the presence of LLV using this test.

* Both in vitro- and in vivo-derived FV complex were assayed for the presence of infectious SFFV using the spleen focus assay and for the presence of LLV using the XC plaque assay as described in Experimental Procedures. SFFV and LLV titer estimates are expressed in FFU and PFU, respectively.

‡ The form of leukemia induced was confirmed histologically using spleen, liver, and lymph node tissue sections as well as peripheral blood smear preparations. Those mice exhibiting erythroid disease were found to be polycythemic with hematocrits ranging from 60 to 80%, while those mice presenting with lymphatic leukemia had hematocrits ranging from 8 to 19%.

§ The spleens from all leukemic mice exceeded a weight of 1 g. From each individual spleen, a 20% wt/vol extract was prepared and the cell-free supernatant fluid was assayed for the presence of SFFV, LLV, or both, as described above. Although each animal was tested separately for SFFV and LLV, the SFFV and LLV titer estimates presented here represent the average titer estimate for all positive leukemic mice in each group.

nally inoculated with either FV-TC or in vivo-grown FV complex can provide a helper function (24) to defective SFFV in vivo and is neutralized by type-specific murine antisera directed against LLV-F.

Discussion

The studies presented in this paper show for the first time extensive growth of Friend SFFV in a nonhematopoietic target cell. Unlike other strains of MuLV which have been successfully grown in monolayer cultures of MEF for extended periods of time and have become attenuated (8–13), the defective SFFV component of the in vitro-derived FV complex, and the LLV-F helper virus did not decrease in leukemogenic potential (Table II).
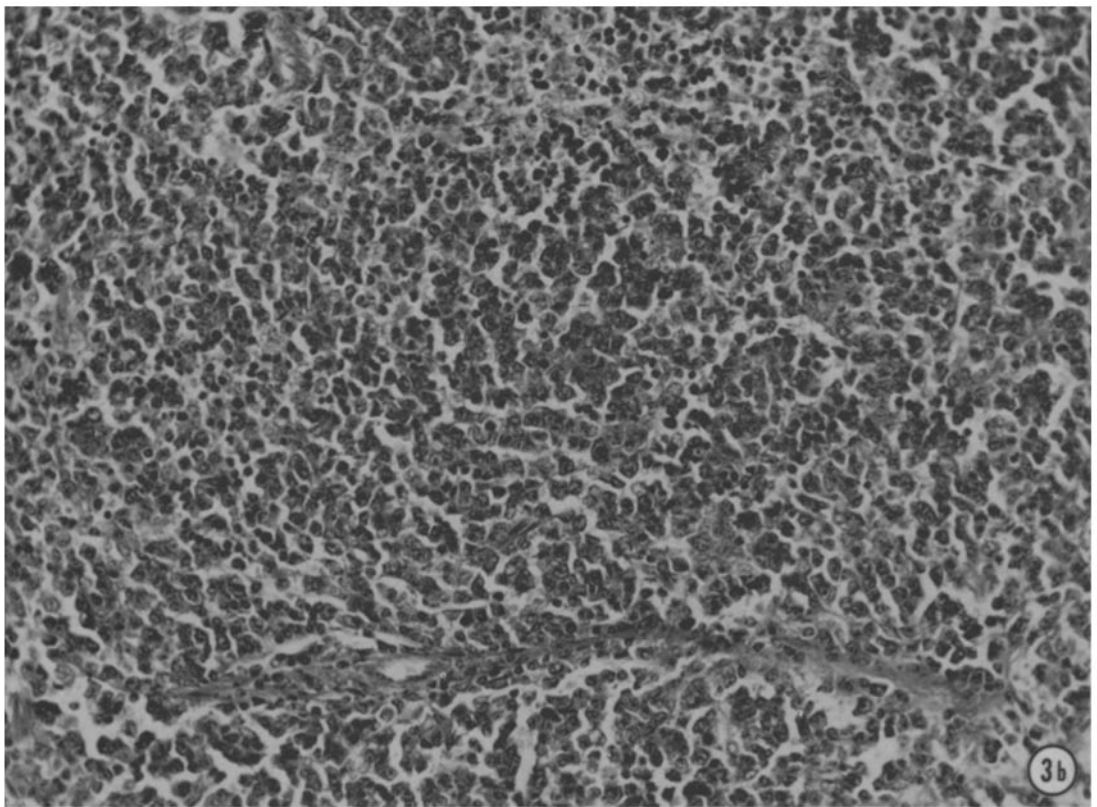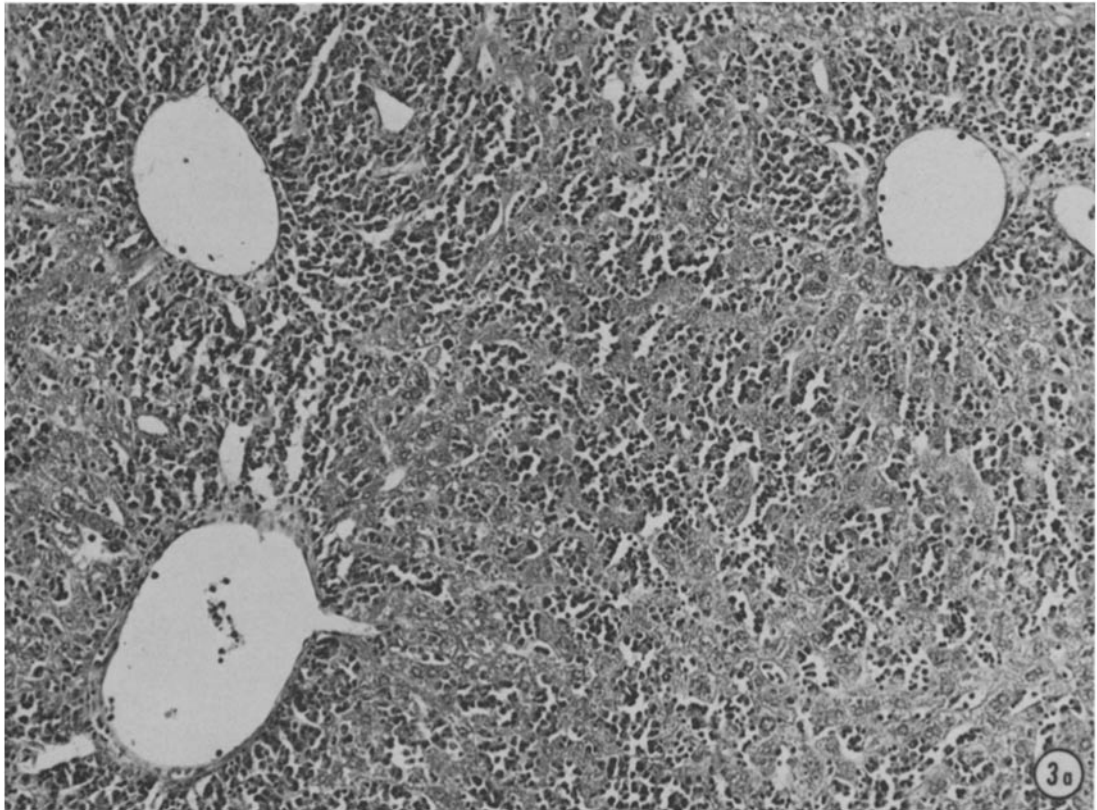
Yoshikura et al. (12) have shown that nonleukemogenic strains of FV (presumably LLV-F only) may be propagated in vitro and are infectious for MEF cultures based on their ability to interfere with infection by murine sarcoma virus (MSV), and to act as a helper for defective MSV. In a similar fashion, the LLV-F obtained from Swiss-FV cells acts as a helper for both defective SFFV and MSV.

A basic question remains unanswered: why is LLV-F, unlike other fibroblastic cell-grown MuLV's, fully leukemogenic after extended growth in NIH Swiss cells? This observation may be due to the continued presence of fully leukemogenic SFFV in this culture system. There may be an intracellular viral interaction between SFFV and LLV-F in nonhematopoietic tissue which allows for the persistence of oncogenic potential in both viruses. An interdependency between these two viruses for replicative events has been described (1, 18, 25), and it is not unreasonable to speculate that complementation of defective and helper virus functions occurs at the level of viral-induced transformation of susceptible target cells as well. A recent report by Greenberger et al. (26) suggests that the endogenous "mouse-tropic" type-C virus of C58 (C58-MuLV) and BALB/c MEF (BALB:virus-1) are able to induce lymphatic leukemia and myeloid metaplasia, respectively, when inoculated into newborn NIH Swiss mice. This in vivo biological function was presumably detected in the absence of other infectious or induced MuLV's. However, it is possible that the appearance of leukemia in C58-MuLV-inoculated mice may be only partially related to the presence of this virus since only 14% of such inoculated mice developed splenomegaly 15 mo after infection. The possible "association" between C58-MuLV infection and leukemia induction might be further defined by comparing the antigenic markers of the leukemic cells recovered to those of the original C58-MuLV as well as to any infectious virus present in or on leukemic cells. It has also been suggested that immunologically activated or induced MuLV's are leukemogenic (27–29). Such spleen cell-derived MuLV genomes (i.e., mouse-tropic XC plaque formers) are most certainly leukemia-associated and may be of etiological importance since 70% of virus-infected mice have been shown to develop lymphoreticular tumors (27).

The availability of in vitro (MEF)-derived FV complex will allow the production of large quantities of purified virus for use in studies concerning the biophysical and biochemical properties of both SFFV and LLV. Further, it may be possible to determine what material is perhaps nonspecifically packaged into both SFFV and LLV-F particles due to their intimate association with erythroid cells in vivo. It has already been shown by Ikawa et al. (30) that host globin messenger RNA's are incorporated into FV particles. The observation that FV-TC is mitogenic for some mouse lymphoid cells has been extended to show that only cells contained in the thymus respond to FV-TC. Spleen and lymph node

FIG. 3. Histopathology of leukemia induced in BALB/c mice by in vitro-derived LLV-F. (a) A low power view of the liver to demonstrate the infiltrate of leukemia cells, principally about the central veins, but also within the hepatic sinusoids. (H & E, ×100). (b) The large leukemic cells with large vesicular nuclei and prominent nucleoli flood the splenic pulp and virtually replace the smaller lymphocytes (seen as small dark interspersed forms). (H & E, ×200).

suspensions do not contain FV-TC-responsive cells. This virus-cell interaction which results in the proliferative response of some lymphoid tissue may allow us to critically investigate the role of various lymphocyte subpopulations in surveillance against mouse leukemia.

Summary

Exposure of NIH Swiss mouse embryo fibroblasts (MEF) to infectious Friend virus (FV) complex [containing defective spleen focus-forming virus (SFFV) and endogenous NB-tropic leukemia-inducing helper virus (LLV-F)] resulted in the productive infection of these cells by both SFFV and LLV-F. Stocks of SFFV derived after extensive growth in this Swiss MEF cell culture system are fully leukemogenic in adult mice for the induction of erythroleukemia and spleen foci. In addition, in vitro-derived LLV-F, when isolated free of SFFV, is fully leukemogenic for the induction of lymphatic leukemia when inoculated into susceptible newborn BALB/c mice. The host range of in vitro-derived FV complex (i.e., FV-TC) for focus formation in vivo is NB-tropic. Unlike in vivo-derived FV complex, FV-TC does not suppress the responsiveness of murine thymocytes to concanavalin A (Con A) in vitro. Rather, FV-TC acts as a mitogen to nonspecifically stimulate the proliferation of BALB/c thymocytes. The mitogenicity of in vitro-derived FV complex is directly associated with the presence of type-C virus particles, is a heat-labile and UV-sensitive property of the virus, and may be primarily due to LLV since equivalent amounts of LLV with or without SFFV present are equally mitogenic. One in vivo passage of FV-TC resulted in the total loss of this mitogenic property with the reappearance of full immunosuppressive properties. This result demonstrates a clear association between in vivo growth of FV and its ability to suppress mouse thymocytes, and suggests that FV complex (SFFV-LLV) is not inherently immunosuppressive for these cells. While the mechanism of this interconversion between immunostimulatory and fully suppressive virus is unknown, both virus markers appear to be dependent upon the presence of infectious FV.

Addendum. Dr. A. Axelrad (University of Toronto, Ontario, Canada) has recently obtained an N-tropic Friend SFFV from an adherent, nonerythroid cell line established from the spleen tissue of FV-infected mice. Although of hematopoietic origin, these cells represent another nonerythroid monolayer culture system producing infectious SFFV (personal communication).

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