Development and Regression of Murine Leukemia Induced by Friend Virus Complex[1] (37307)

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(Introduced by W. P. Sawyer)

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The responses of mice to infection by Friend virus complex (FV) depend on the genome of both the virus (1–3) and the host itself (4–11). Different strains of mice vary in susceptibility to the spleen focus-forming virus (SFFV) (12) of FV (4, 5). Although the kinetics of the replication of SFFV depend on the dose of inoculating virus (13), little attention is given to the effect of dose on the outcome of the disease. This report describes two experimental factors that influence the development and the regression of FV-induced leukemia in mice. These are: (a) the size of the inoculating dose and (b) the strain of mice.

Materials and Methods. Inbred A/J, A/J × C57BL/6J ($AB6F_1$), and their reciprocal hybrids, and random bred Swiss mice of both sexes, aged 6 to 10 wk, were used. The FV stocks which had undergone at least five cell-free passages in Swiss mice were injected intravenously into A/J and $AB6F_1$ and their reciprocal hybrids. At intervals, spleens were removed from 4 to 12 mice and a suspension was prepared in ice-cold Eagle's medium (Grand Island Biological Co., Buffalo, NY) as previously described (14). A spleen(s) that differed markedly in size from the majority was processed separately. The spleen focus assay method (15) was used to quantitate SFFV in the homogenized spleen cell suspensions (Sorvall, Model 2-6100, at top speed for 10 min); six to eight Swiss mice were used in each assay. To measure SFFV-induced tumor cells, infected spleen cells were washed three times in cold Eagle's medium to remove extracellular virus and injected in five to eight $AB6F_1$ and their reciprocal hybrid mice (14).

Results and Discussion. To determine the effect of the inoculating dose of FV on leukemia development and regression, three doses of FV were injected into groups of A/J mice. SFFV increased exponentially for 5 days in the spleens of A/J mice injected

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TABLE I. Recovery of SFFV and of SFFV-Induced Tumor Cells and the Total Number of Cells from the Big Spleens of A/J and $AB6F_1$ Hybrid Mice.

Strain of mice	Dose of virus (FFU)	Time after injection (day)	Proportion of mice with big spleen	Recovery of		
				FFU/spleen[a]	TCFU/spleen[b]	Total no. of cells/spleen
A/J	0.5	33	3/10	$8.8 \times 10^4 \pm 1.7 \times 10^4$	ND[c]	4.3×10^9
		47	2/10	$1.5 \times 10^5 \pm 1.1 \times 10^5$	$3.5 \times 10^4 \pm 0.7 \times 10^4$	1.4×10^9
		61	4/8	$1.7 \times 10^6 \pm 0.4 \times 10^6$	$>10^5$	3.3×10^9
$AB6F_1$	500	61	1/9	<17	$5.0 \times 10^3 \pm 0.2 \times 10^3$	4.3×10^9

[a] Values represent mean ± standard error of 6 to 8 assay mice.
[b] Values represent mean ± standard error of 5 to 8 assay mice.
[c] ND = Values were not determined.

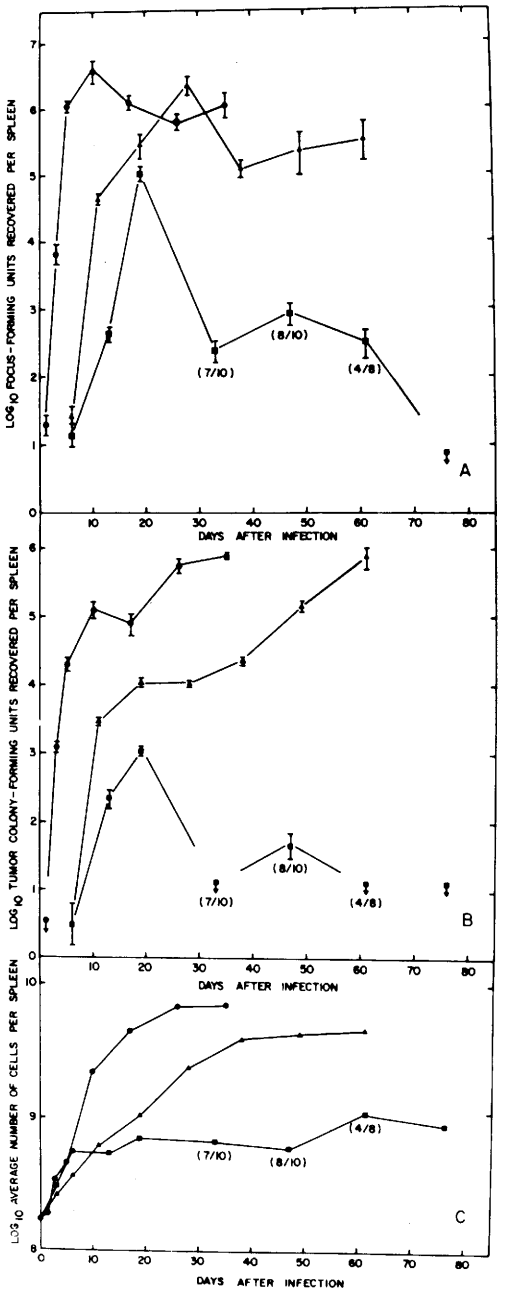

FIG. 1. Increase of SFFV, of SFFV-induced tumor cells, and of total number of spleen cells in A/J mice after the injection of 5000 (●), 5 (▲) and 0.5 (■) FFU of FV. Mean ± standard error of the number of FFU and of TCFU and the mean of the total number of cells recovered from the spleens at each interval are shown. Unless noted, values are those of all mice. Exceptions were on Days 33, 47, and 61 when mice with grossly enlarged spleens were examined separately.

with 5000 FFU of FV and reached the maximal titer on Day 10 (Fig. 1A). The increase of virus was slower in mice injected with 5 FFU of FV, but the maximal titer of the virus was similar ($p > 0.3$). High titers of the virus were maintained in these mice, and most mice died. The titer of the virus in mice injected with 0.5 FFU of FV reached its peak on Day 19. Thereafter, the amount of virus decreased in 73% of the mice (25 of 34; Fig. 1A). In the few (9) A/J mice that had very large spleens, high titers of virus persisted (Table I). No virus was detected in six mice examined 76 days after inoculation. Virus was not detected on Day 76.

In the early period, the growth of SFFV-induced tumor cells in the A/J mice inoculated with FV paralleled that of the SFFV (Fig. 1B). Later, the number of tumor cells continued to increase in mice injected with 5000 and 5 FFU, but with the exception of the few mice with large spleens (Table I), tumor cells were not detected in mice injected with 0.5 FFU. The absence of both virus and tumor cells in A/J mice 2.5 mo after injection indicated the regression of leukemia in these mice. The possibility for regression, thus, seems dose dependent; the large inoculum of FV used in other work (11) may have accounted for failure to observe regression of the disease.

In mice with persistent leukemia, *i.e.*, those that received the larger doses and those few that had persistent gross splenomegaly among the mice infected with the lowest dose (Table I) the number of spleen cells increased (Fig. 1C). In mice that had a regression of disease, the number of cells increased fivefold and was maintained at that level. The persistent increase in spleen cells in the absence of SFFV and SFFV-induced tumor cells is unexplained, but could relate to (a) differentiation of SFFV-induced tumor cells along the the erythrocytic line (16, 17), (b) cells which were involved in an immune response, and (c) spleen cells influenced by the lymphatic leukemia virus that is a part of the Friend leukemia virus complex and has a longer incubation time than the SFFV (18).

The $AB6F_1$ and their reciprocal hybrid

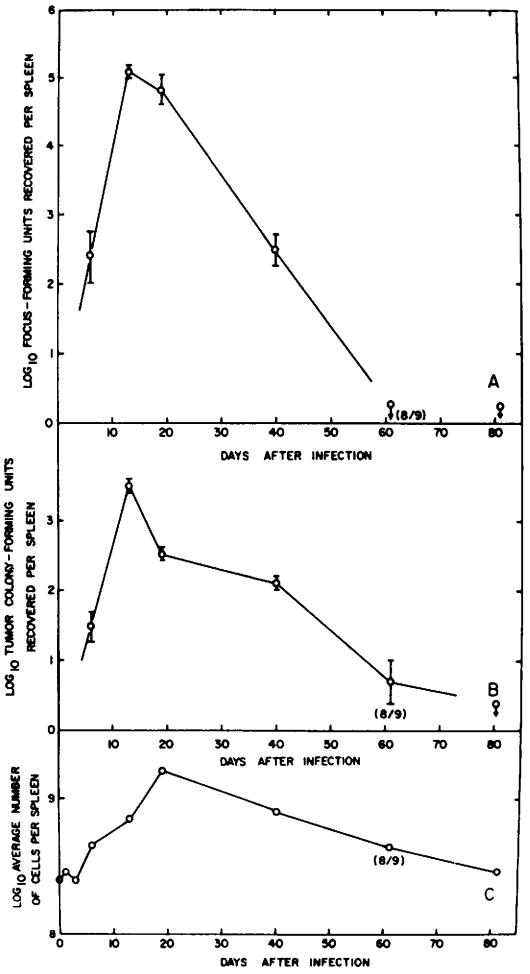

FIG. 2. Increase of SFFV, of SFFV-induced tumor cells, and of the total number of spleen cells in AB6F$_1$ and their reciprocal hybrid mice after the injection of 500 FFU of FV. Mean ± standard error of the number of FFU and of TCFU and the mean of the total number of cells recovered from the spleens at each interval are shown. On Day 61, one mouse had a grossly enlarged spleen and was examined separately.

mice were about 40 times more resistant to FV than the A/J mice (Table II). After injection of 500 FFU of FV, AB6F$_1$ mice rapidly developed leukemia, but the leukemia regressed (Fig. 2A and B). The patterns of increase of SFFV and of SFFV-induced tumor cells were similar. The number of spleen cells increased about 7-fold and gradually declined to normal level thereafter

TABLE II. Number of Spleen Foci in A/J and AB6F$_1$ Mice Injected with Different Amounts of Friend Virus Complex.

Dilution of virus	Foci/spleen	
	A/J mice[a]	AB6F$_1$ mice[b]
10^{-1}	ND[c]	27.8 ± 3.1
10^{-2}	C	0.6 ± 0.4
10^{-3}	6.3 ± 1.4	0
10^{-4}	0.8 ± 0.2	ND

[a] Values represent mean ± standard error of 9 to 12 mice.

[b] Values represent mean ± standard error of 5 to 6 mice.

[c] ND = values were not determined; C = the spleen foci were confluent.

(Fig. 2C). One AB6F$_1$ mouse had distinct splenomegaly associated with tumor cells in the spleen two months after the injection of FV (Table I), but the spleen size of all other mice was normal.

The regression of FV-induced leukemia in mice that is described herein occurred earlier than that reported recently (1, 2). Perhaps the difference relates to the quantitation of the inocula and of the disease; only spleen weight and histologic examination were used by other investigators (1, 2).

Summary. Injection of a large dose (5000 FFU) of Friend virus complex (FV) lead to rapid development of leukemia and to death in A/J mice. Leukemia developed at a slower rate in A/J mice inoculated with 5 FFU of FV. A very small dose (0.5 FFU) of the same virus caused slow development of leukemia that later regressed in most A/J mice (73%). In (A/J × C57BL/6J) F$_1$ and their reciprocal hybrids, leukemia developed rapidly after the injection of 500 FFU, but regressed in 97% of the mice.

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