The Potential for Leukemia Regression in BALB/c Mice[1] (35506)

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It has been demonstrated that the leukemia in Swiss mice induced by a specific strain of virus regresses spontaneously (1). The disease induced by this virus strain is similar to that initiated by Friend or Rauscher leukemia virus (2, 3). The leukemia differs from that initiated by conventional virus strains in that it appears to be self-limiting. The characteristic massive splenic response in a high percentage of inoculated mice is not followed by splenic rupture and death, but rather by a return of the spleen to relatively normal structure (4), by a marked decrease in the proliferation of infectious virus, and by the apparent recovery of a significant number of inoculated mice (1).

It was observed that the disease in BALB/c mice, unlike that in other mouse strains, did not appear to regress (9). Since leukemia does not regress until a fixed time period after virus infection (5), leukemia in BALB/c mice might regress if the mice could survive long enough for the regression to be expressed. Alternatively, it could be postulated that, as a function of the genetic character of the host, leukemia regression in this mouse strain does not indeed occur. The present study distinguished between these hypotheses and established the latter to be true for BALB/c mice.

Materials and Methods. Using protocols previously described (6), cell-free virus preparations were prepared from 20% suspensions [in saline (w/v)] of spleens from leukemic Swiss ICR/Ha mice previously inoculated with conventional or regressing Friend leukemia virus (1). Virus was stored at −70° in sealed ampuls. Unless otherwise specified, mice were inoculated ip with 0.5 ml containing 100 ED_{50} doses of the appropriate virus stock. An ED_{50} leukemia dose is defined as that dose of virus which will produce characteristic Friend leukemia in 50% of inoculated Swiss mice within 25 days.

Random bred ICR/Ha Swiss mice were from the colony maintained in our laboratory. Inbred BALB/c mice were purchased from Jackson Laboratory, Bar Harbor, Maine. In all cases, 5-week-old male mice were used.

Animals were examined for splenic enlargement at weekly intervals after inoculation. Diagnosis of leukemia was confirmed by gross and microscopic examination with criteria and protocols previously reported (7). Deaths were recorded daily.

Results. Groups of mice were inoculated with a leukemogenic dose of either conventional or regressing Friend leukemia virus.

Table I shows that both BALB/c and Swiss mice were susceptible to regressing (RFV) and conventional Friend virus (FV). The initial leukemogenic response, as measured by incidence and latent period, to FV and to RFV were similar in both mouse strains. The initial splenomegaly associated with Friend leukemia (2, 8), was observed in all four experimental groups (Table I).

In 75% of the Swiss mice inoculated with RFV, the leukemia regressed by 56 days postinoculation. In contrast, leukemia regression

[1] This investigation was supported by U.S. Public Health Service Grant CA-06711 from the National Cancer Institute.

[2] Research Career Development Awardee (1-K3-CA21773) of the National Cancer Institute.

[3] Supported by U.S. Public Health Service Training Grant GM 00983.

TABLE I. Leukemia Regression in Swiss and BALB/c Mice.

Mouse strain	Virus[b]	Leukemia incidence[a]			
		No. leukemic/no. inoculated	Median latent period (days)	Range	% Regression[c]
Swiss	FV761	10/10	14	14–21	0
	RFV748	8/10	14	14–21	75
BALB/c	FV761	9/9	12	12–21	0
	RFV748	7/10	21	12–21	0

[a] Swiss and BALB/c mice were observed for 184 and 240 days, respectively.

[b] FV, conventional, nonregressing Friend leukemia virus; RFV, regressing Friend leukemia virus.

[c] Number of mice which were leukemic by day 21 and were negative by day 56 (spleen weights less than 300 mg) divided by the number of leukemic mice × 100.

was not observed in any of the BALB/c mice inoculated with the same virus strain. In comparing deaths in this experiment it was observed that as early as 49 days, when only 2 of the 18 leukemic Swiss mice were dead, 9 of the 16 BALB/c mice were already dead of leukemia. This suggested that BALB/c mice, as a consequence of their greater sensitivity, might not survive long enough for leukemic regression to occur.

To determine the time parameters for regression, Swiss mice were inoculated with 5 separate stocks of RFV and the incidence and time to leukemic regression were determined for each group. As shown in Table II, the response to the various virus stocks was relatively uniform. The mean time interval between virus inoculation and regression of the resultant disease was 41 days (1 SD = 9 days). Thus, significant numbers of BALB/c mice might be dead before the time interval at which the leukemia was observed to regress. To determine if this were the case, it was necessary to extend the life expectancy of leukemic BALB/c mice past the point where, in other mouse strains, regression is known to occur.

TABLE II. Time Parameters for Regression in Swiss Mice.

Virus	No. regressed/no. leukemic	Time to regression (days)[a]	
		Mean ± SD	Median
RFV723	5/6	36.6 ± 13.9	28.0
RFV733	4/4	40.0 ± 0.0	40.0
RFV741	6/8	38.0 ± 4.4	40.0
RFV748	5/8	50.4 ± 10.2	42.0
RFV748	6/8	43.1 ± 9.4	38.5
RFV767	6/9	39.6 ± 7.7	35.0

[a] Mice were observed for 120 days.

As shown in Table III, when BALB/c mice were inoculated with varying doses of RFV or FV, both the time interval to leukemic development and to death were inversely proportional to the virus dose. A 20-fold reduction in virus dose (100 to 5 ED_{50} of RFV) significantly delayed the onset of leukemia and the resulting death. Leukemic mice inoculated with 5 ED_{50} of RFV were alive until 90 to 160 days postinoculation. Despite the prolongation of life, regression was not observed in any of the BALB/c mice inoculated with the regressing leukemia virus strain.

As shown in Table III, the leukemogenic response of BALB/c mice to RFV and to FV was essentially the same with respect to both initial response and to the time course of the disease.

A comparison of the median time to leukemia incidence with the median time to death for both RFV and FV (Table III) suggests that the time period between the development of leukemia and death is relatively constant (58 and 56 days for RFV and FV, respectively). This time interval, unlike time to leukemia incidence, appears to be dose independent.

Discussion. An understanding of the mechanisms underlying the spontaneous regression of leukemia offers potential insight

TABLE III. Leukemogenic Response of BALB/c Mice to Varying Doses of Conventional and Regressing Friend Virus.

Virus	ED_{50}[a]	Leukemia incidence				Mortality		
		No. leukemic/no. inoculated	Median (days)	Range	% Regression	No. dead/no. leukemic	Median (days)	Range
	1	0/10	—	—	—	0/10	—	—
RFV[b]	5	6/10	49	36–148	0	5/6[d]	111	90–160
748	10	9/10	36	28–56	0	7/9[d]	98	77–157
	100	9/10	21	21–28	0	8/9[d]	71	44–131
	1	4/10	53	42–90	0	4/4	125	96–148
FV[c]	5	6/10	42	36–105	0	5/6[d]	77	75–131
761	10	10/10	28	21–36	0	10/10	92	42–130
	100	10/10	21	21–28	0	10/10	74	36–141

[a] As determined in Swiss mice.

[b] Regressing strain of Friend virus.

[c] Nonregressing strain of Friend virus.

[d] At 154 days, surviving mice were alive and leukemic with spleen weights estimated by palpation to be greater than 2 g (normal spleen wt = 0.1 to 0.2 g).

into both the etiology and the effective control of the disease. In earlier studies it was demonstrated that the regression of leukemia was, at least in part, a property of the viral genome (1). In addition to the appropriate viral genome, the presence of those environmental factors that permit the mouse to live long enough for regression to be expressed were also required (5).

It was demonstrated that BALB/c mice die of leukemia earlier than do Swiss mice. This suggested that BALB/c mice, as was observed for young Swiss mice (5), might be dying before leukemic regression could occur. Since leukemia regression was not seen in BALB/c mice it was necessary to determine the time parameters for regression in Swiss mice and to assume that the time parameters would be similar for both mouse strains.[4] It was evident that by 41 days, the mean time for the occurrence of regression (Table II), that a significant proportion of BALB/c mice infected with 100 ED_{50} of Friend virus were dead of, or severely and perhaps irreversibly afflicted with, Friend leukemia. By reducing the infecting dose (Table III), it was possible to delay the onset of leukemia and the resultant time to death considerably beyond the time interval at which regression was observed to occur. Half of the leukemic BALB/c mice in this group were still alive by 111 days, long after the interval during which regression is observed to occur in other mouse strains. The complete absence of leukemia regression in these mice strongly suggests that regression cannot occur in BALB/c mice, and that this failure to regress is not a function of environmental factors that prohibit the expression of regression, but rather, that it reflects some specific genetic character of the host.

Since leukemia regression depends on the appropriate host response to a specific viral genome, absence of leukemia regression could result from: nonrecognition of the specific aspect of the viral genome related to regression; the inability of the host to respond in an appropriate fashion; or the development of a leukemia which is different from the disease in other hosts, in that it is not sensitive to those host factors which elicit leukemia regression. Studies in progress are aimed at differentiating among these alternatives.

Summary. The spontaneous regression of leukemia observed in other mouse strains does

[4] In a subsequent study on regression in other mouse strains it was observed that the time parameters for regression were similar to those for Swiss mice.

not occur in BALB/c mice. Regression was not observed even when the onset of leukemia and the resultant death were delayed beyond the time period when leukemia regression is known to occur in other mouse strains. It is suggested that the observed absence of leukemia regression in BALB/c mice reflects some specific genetic character of the host.

The authors thank Mr. Rodney Clymer for his excellent assistance.

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Received Nov. 24, 1970. P.S.E.B.M., 1971, Vol. 137.