Murine C-type RNA Virus from Spontaneous Neoplasms: in vitro Host Range and Oncogenic Potential

Abstract. *Strain BALB/c mice harbor at least two host range variants of murine leukemia virus. One variant, which is host-cell tropic, is the predominant isolate from neoplastic tissues and produced lymphoreticular neoplasms when injected into BALB/c newborn mice. A second variant, which is isolated throughout life, grows poorly in host embryonic cells in culture and was not associated with lymphoreticular neoplasm induction when injected into newborn BALB/c mice.*

We have evaluated the incidence of C-type RNA viruses of each of the two tropisms present in a variety of spontaneous neoplasms of the BALB/cCr mouse strain and have determined the association between the virus type and its oncogenic potential.

Wild-type (naturally occurring) strains of murine C-type RNA virus (commonly referred to as murine leukemia virus—MuLV) have preferential growth capability on either NIH Swiss embryonic (NIH-E) or BALB/c embryonic (B/c-E) cells in culture. Those strains growing preferentially on NIH-E cells are termed N-tropic and those on B/c-E cells, B-tropic (*1, 2*). Embryonic cells from the various mouse strains tested were all shown to preferentially support the replication of B-tropic or N-tropic virus and were therefore termed B-type or N-type, respectively (*1, 2*). The embryonic cells from the BALB/c mouse are B-type and this strain has become the prototype B strain. The BALB/c mouse, however, harbors both N- and B-tropic viruses naturally (*1, 3, 4*) with a predominance of N-tropic virus (>90 percent of isolates—16 of 17) in mice under 12 months of age and a predominance of B-tropic virus in late life (>60 percent of isolates from mice over 24 months of age—11 of 22) (*4*).

This change with aging from a predominance of N-tropic to one of B-tropic was of interest since the incidence of spontaneous neoplasms increased progressively with age (*5*).

Spleen and tumor tissues were obtained from mice with spontaneous neoplasms that occurred in a large untreated holding colony. The mice, their natural neoplasia incidence, and types of neoplasms occurring have been described (*5*). Tissues were collected when the mice were killed and stored at −198°C before testing. All tissues were processed and used the same day. These tissues were prepared for cell-free inoculation by the Moloney procedure (*6*). The pellets obtained after clarification and centrifugation at 30,000*g* for 1 hour (*6*) were suspended to 3.0 ml in 0.05*M* sodium citrate (*p*H 6.8) or to a final dilution not exceeding 1 : 20, based on the original weight of the tissue used for extraction. After the pellets were suspended, 10 to 20 BALB/cCr mice (<72 hours old) were randomly selected and inoculated with 0.05 ml of each given preparation. Preparations from spleens were inoculated intraperitoneally, and preparations from tumors were inoculated subcutaneously and intraperitoneally into separate groups of mice. Duplicate, secondary, petri dish cultures of B/c-E and NIH-E cells were inoculated with 0.05 ml of each specimen and maintained, harvested, passaged, and tested for MuLV group-specific (gs) antigen by the complement fixation test for MuLV (CoMuL) as described (*3, 4*). As an additional test for virus isolation and as a measure of growth potential in vivo, spleens from three to five mice from each inoculated and uninoculated control group were obtained 63 days after inoculation, and the spleens [10 percent (weight to volume) extracts] tested for MuLV-gs antigen by the spleen antigen complement fixation test (SPAT) (*7*). A given inoculum was considered SPAT positive when at least two of the sampled spleens were positive and the controls were negative. If only one of the spleens sampled was positive, it was passaged into newborns and tested again 21 days later. Mice were observed weekly for 2 years, and all animals becoming moribund or having a suspected neoplasm were killed and processed histologically. Lymphoreticular (LR) neoplasms (including lymphocytic leukemia, lymphosarcoma, reticulum cell neoplasms of type A and B) were classified according to Dunn (*8*). All other tumors were classified according to standard histopathologic descriptions.

Tumor and spleen specimens were processed and inoculated into cell cultures of B/c-E and NIH-E fibroblasts and into BALB/cCr suckling mice on the same day. A predominance of B-tropic virus as compared to N-tropic virus was observed among isolates from neoplastic tissues giving positive SPAT tests (Table 1). Of all inocula yielding virus by the CoMuL test, neoplastic spleens (LR neoplasm donors) yielded 10 isolates on B/c-E cells only, 3 isolates on NIH-E cells only, and 3 isolates on both cell types. Similarly, solid tumor tissue gave 19 isolates on B/c-E cells only, 2 isolates on NIH-E cells only, and 1 isolate on both cell types. Spleens (histologically normal, except in two instances) obtained from animals bearing solid tumors also had a predominance of B-tropic virus, 9 isolates on B/c-E cells only, 5 isolates on NIH-E cells only, and 2 isolates on both NIH-E and B/c-E cells. This difference was not as marked, however, as that observed in neoplastic tissues. Studies of spleens from normal, non-tumor-bearing, age-matched control animals have not shown this extreme predominance of B-tropic virus (*4*).

When the assay in vivo (SPAT) was compared to the assays in vitro (CoMuL), the incidence of MuLV isolation was similar for all tissues tested. The incidence by SPAT was 18 of 46 (39 percent), 18 of 40 (45 percent), 15 of 29 (52 percent) for spleens from animals bearing solid tumors, from solid tumor tissues, and from LR neoplasms, respectively, as compared to incidences by the CoMuL test of 16 of 62 (26 percent), 22 of 50 (44 percent), and 16 of 29 (55 percent), respectively (Table 1). Although the incidence rates by these two methods were similar, the same result was not always obtained simultaneously by the two methods. Of the 54 specimens that were CoMuL positive, 37 (69 percent) were SPAT positive. However, when the CoMuL data were divided into B-tropism and N-tropism, the relation between B-tropism and SPAT isolation was evident. Of 43 B/c-E isolates there was a concomitant SPAT positive in 35 (81 percent) of the instances, whereas of the 16 NIH-E isolates there was a concomitant SPAT positive in 7 (44 percent) of the instances (5 of these 7 were isolated on both cell types). Conversely, of the total of 51 specimens that were SPAT positive, 37 (73 percent) were CoMuL positive

and 30 of these were B-tropic, 2 were N-tropic, and 5 were isolated on both cell types.

As shown in Table 2, induction of LR neoplasms by the spleen and tumor inocula was further assessed on the basis of the combinations of factors measured in Table 1. Specimens yielding a combination of SPAT positive and B/c-E positive were highly leukemogenic (185 of 478, or 38.7 percent); however, those yielding the combination SPAT positive and NIH-E positive (2 of 14, or 14 percent) did not significantly exceed the uninoculated control rate (23 of 231, or 10 percent; not shown in Table 2). A positive SPAT alone yielded a significant increase in LR neoplasms (51 of 211, or 24.2 percent), whereas a positive CoMuL test alone on B/c-E (12 of 92, or 13.0 percent) or NIH-E (13 of 106, or 12.3 percent) without a concomitant positive SPAT did not cause a significant increase above the uninoculated control rate (23 of 231, or 10 percent). When the SPAT and both cell types were positive in the CoMuL assay a highly significant rate of LR neoplasm induction occurred (30 of 79, or 38 percent).

Mean latent periods for LR neoplasm induction by MuLV positive inocula (465 days) were significantly less than those for MuLV negative inocula (545 days) or for the uninoculated controls (634 days). The latent period differences between the MuLV negative inocula and the uninoculated controls seem to indicate acceleration by undetected MuLV without increased incidence rates.

The comparative growth capabilities in vitro of N- and B-tropic MuLV have been reported (*1*, *2*). These reports showed that high multiplicities of infectious virus were required for the N- and B-tropic variants to propagate on the B/c-E and NIH-E resistant cells, respectively. Thus, with the low titers of MuLV (80 percent of positives isolated only after two to three "blind" passages) in the inocula used, few isolations were made on both cell types (4 percent), and this barrier may be even higher in vivo. The predominant MuLV variant isolated in cell culture from the spleen or tumor tissues of mice bearing spontaneous neoplasms was B-tropic (38 of 51, or 75 percent isolated on B/c-E cells only) as opposed to N-tropic (10 of 51, or 20 percent on NIH-E cells only) (Table 1). This contrasts with results for spleen tissues (histologically normal) of normal mice from the same holding colony (*3*) as the donor mice, where 16 of 51 trials (31 percent) were made on NIH-E cells only, 16 of 51 (31 percent) on B/c-E cells only, and 19 of 51 (37 percent) on both NIH-E and B/c-E cells. The donor mice for this study were 18 to 30 months of age; those sampled from the holding colony were 19 to 32 months of age.

The ability to grow in the spleen (SPAT) and B-tropism in cell culture were significantly associated. This was not surprising since a B-tropic MuLV has been defined by Hartley (*1*) and Pincus (*2*) and their co-workers as a MuLV that propagates preferentially on B/c-E cells as opposed to NIH-E cells. However, this differentiation has not heretofore been extended to the in vivo system. The sensitivity of BALB/c spleen cells in vivo to the growth of B-tropic virus was further reflected in the induction of LR neoplasms by cell-free preparations that yielded B-tropic virus in cell culture. However, B-tropism without sufficient titer to produce a positive SPAT was not adequate to produce increased rates of LR neoplasms (Table 2). No relation between N-tropism and LR neoplasm induction was found. Although the N-tropic variant comes from the BALB/c mouse, it propagates poorly in the cells of its host in cul-

Table 1. Isolation of murine leukemia virus (MuLV) from spleen and tumor tissues of BALB/cCr mice bearing spontaneous neoplasms. Donors of solid tumors included those with primarily sarcomas, carcinomas, hemangioendothelioma, myoepithelioma, and mammary tumors. Lymphoreticular neoplasms included leukemias, lymphosarcoma, and reticulum cell neoplasms. The SPAT result indicates the replication of MuLV in vivo as detected in spleen extracts (10 percent, weight to volume) from three to five mice sampled at random from each inoculum group. The CoMuL was obtained in the following way: 0.5 ml of each inoculum was pipetted onto plate cultures of NIH Swiss and BALB/c mice. After 21 days, cells were harvested and tested for the gs antigen of MuLV. Three passages were completed before a test was considered negative; NT, not tested.

Tissue	SPAT	CoMuL (No.)			
		Negative	Positive		
			NIH-E only	B/c-E only	B/c-E and NIH-E
		Solid tumor donors			
Spleen	Positive	8	1	7	2
	Negative	23	3	2	0
	NT	15	1	0	0
Tumor	Positive	4	0	14	0
	Negative	17	2	3	0
	NT	7	0	2	1
		Lymphoreticular neoplasm donors			
Spleen	Positive	2	1	9	3
	Negative	11	2	1	0
	NT	0	0	0	0

Table 2. Correlation of the presence of MuLV in cell-free spleen and tumor tissue preparations (derived from BALB/cCr mice bearing spontaneous neoplasms) with the induction of lymphoreticular neoplasms. Results are expressed as the ratio of the number of mice succumbing to lymphoreticular neoplasms after inoculation with cell-free preparations to the number of mice surviving past weanling age and receiving inocula with the specified characteristics. Percentages appear in parentheses. The presence of MuLV was determined for each inoculum by the CoMuL test for growth on both BALB/c and NIH Swiss embryonic fibroblasts in culture and for most specimens by SPAT for growth in vivo in the spleens of inoculated mice sampled from each test group; NT, not tested.

Tissue	SPAT	CoMuL			
		Negative	Positive		
			NIH-E only	B/c-E only	B/c-E and NIH-E
		Solid tumor donors			
Spleen	Positive	27/120(23)	2/14(14)	52/123(43)	14/33(42)
	Negative	39/369(11)	5/40(13)	3/27 (11)	
	NT	17/105(16)	2/13(15)		
Tumor	Positive	15/62 (24)		75/218(34)	
	Negative	22/226(10)	4/32(13)	3/48 (6)	
	NT	9/60 (15)		3/17 (18)	3/4 (75)
		Lymphoreticular neoplasm donor			
Spleen	Positive	9/29 (31)	0/13(0)	57/137(42)	16/46(35)
	Negative	16/167(10)	4/34(12)	6/17 (35)	

ture (*1*). It cannot be said that the N-tropic variant of MuLV from the BALB/c mouse is nononcogenic in the BALB/c host because it is possible that with high doses neoplasm induction could take place. In order to maintain the "natural state" of the MuLV in the neoplasms used we elected, however, not to attempt passage to build up titer and chance artificial selection or alteration of the C-type RNA virus genome (or genomes) that might be present.

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Viability versus Fecundity Selection in the Slender Wild Oat, Avena barbata L.

Abstract. *Estimates of relative selective values of different genotypes in natural populations of predominantly self-pollinated plants have consistently shown a net heterozygote advantage. Heterozygote advantage is further analyzed in the present study by estimating components of selective values corresponding approximately to viability and fecundity. The results show that a higher proportion of heterozygotes than of homozygotes survive from seedling to adult stages and that this viability selection accounts for the overall excess of heterozygotes.*

A central problem of experimental population genetics is the estimation of selection intensities in natural populations. The difficulties associated with such measurements are manifold and their paucity has been cited as one of the major shortcomings of the field (*1*).

Annual plant species offer a number of technical advantages for the estimation of selection. Their chief advantage is monoecism featuring simple mating systems that can be described precisely, thus satisfying the assumptions of simple selection models. Other advantages are ease of collection, and sedentary habit which allows precise identification of population units and accurate description of ecological relationships. Thus it is not surprising that annual plants have been the basis of several studies to measure selection intensities (*2*). Each of these studies has revealed intense selection featuring reproductive advantage of heterozygotes, which was often twice that of homozygotes. The present report describes a preliminary effort to apportion this intense selection over life-cycle stages in the slender wild oat species, *Avena barbata* L.

The population studied occupies a 10-acre site (1 acre = 0.4047 hectare), near Geyserville, California, which supports a vigorous stand of nearly pure *A. barbata* numbering several millions of individual plants. In a previous study of this site (*3*) significant excesses of heterozygotes were found for four esterase loci (E_1, E_4, E_9, and E_{10}), whose assay techniques and formal genetics have been described (*4*). The present study was based on three of these loci (E_4, E_9, and E_{10}).

To partition estimates of selective values into components corresponding approximately to viability and fecundity, census data taken prior to and following the operation of viability selection are required (*5*). In this study, census data were taken on seedlings in the fall of 1969 (generation n prior to viability selection), on adults in the spring of 1970 (generation n after viability selection), and on 1970 seedlings (generation $n+1$ after generation n fecundity selection but prior to generation $n+1$ viability selection). Two weeks after the first wetting rain in the fall of 1969, 140 randomly chosen *A. barbata* seedlings collected in nature were transplanted individually into peat pots. Selection was minimized by growing these seedlings under optimal conditions in a greenhouse for a month and then transplanting them into a garden plot in which conditions were also optimal. At maturity, selfed seeds were harvested from 66 randomly chosen plants among the 133 survivors, and the esterase genotype of each adult plant was inferred from electrophoretic assays of six of its seedling progeny [probability of correct identification $>.995$ (*6*)]. Estimates of genotypic frequencies in 1969 seedlings were made from these data. Genotypic frequencies in 1970 adults were obtained from assay of seven seedlings grown from seed harvested from individual plants in nature at maturity in the spring of 1970. An estimate of genotypic frequencies for 1970 seedlings was obtained from a census of seedlings grown from seeds collected from the ground in the late summer of 1970, before the first fall rain.

Table 1 gives estimates of allelic

Table 1. Allelic frequencies, fixation indices, and their standard errors. N refers to numbers of families examined for the 1969 seedling (S69) and 1970 adult (A70) censuses and to numbers of seedlings examined for the 1970 seedling census (S70).

Census	N	Allele 1	Allele 2	$\hat{F}$
		Locus E_4		
S69	66	0.303	0.697 ± 0.05	0.856 ± 0.07
A70	85	.329	.671 ± .05	.841 ± .06
S70	200	.300	.700 ± .03	.893 ± .03
		Locus E_9		
S69	66	0.227	0.773 ± 0.05	0.914 ± 0.06
A70	101	.208	.792 ± .04	.760 ± .08
S70	211	.206	.794 ± .03	.868 ± .05
		Locus E_{10}		
S69	66	0.204	0.796 ± 0.05	0.861 ± 0.08
A70	101	.232	.768 ± .04	.806 ± .07
S70	212	.172	.828 ± .02	.884 ± .04