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Host-Range Restrictions of Murine Leukemia Viruses in Mouse Embryo Cell Cultures

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Murine leukemia virus strains fall into three categories with respect to their ability to propagate in cells of National Institutes of Health (NIH) Swiss and BALB/c mouse embryos. Cultures of NIH cells are 100- to 1,000-fold more sensitive to "N-tropic" strains than BALB/c cell cultures, but are 30- to 100-fold less sensitive to "B-tropic" strains. Some virus strains (dually tropic or "NB-tropic") propagate equally well in both cells. M-MSV pseudotypes show the host-range characteristics of the virus supplying the envelope, both in vitro and in vivo. The host-range characteristics appear to be genetically determined and could not be explained by host-induced modification or virus mixtures. There was no correlation between host range and Gross-AKR or FMR serotype.

In studies of the viruses of the avian and murine leukemia-sarcoma complexes, a number of observations have focused attention on the importance of host-range variants. First, a number of sarcomas initially thought to be non-infectious have been found to yield virus with a host range completely different from that of the initiating virus (1, 6, 8, 12, 14, 15; G. Kelloff et al., *unpublished data*). Second, the different serological types of avian leukosis-sarcoma viruses can be clearly distinguished by the sensitivity or resistance of cells derived from genetically defined chickens (11, 17). For example, C/A cells are essentially completely resistant to serotypes of the A subgroup while fully sensitive to type B strains, whereas C/B cells show the reciprocal pattern (17). In the case of the avian leukosis viruses, the host-range restriction is at the level of viral penetration or uncoating (2, 13).

This report describes host-range differences between murine leukemia virus (MuLV) strains in their ability to propagate in mouse embryo cell cultures.

MATERIALS AND METHODS

Tissue cultures. Cultures of 15- to 17-day-old National Institutes of Health (NIH) Swiss and BALB/cN mouse embryos (ME) were prepared as previously described (4). Secondary cultures were planted in McCoy's 5A (modified) medium with 10% unheated fetal calf serum, penicillin (50 μg/ml), streptomycin (50 μg/ml), and neomycin (100 μg/ml). At the time of inoculation and for future maintenance, cultures were changed to medium consisting of Eagle's minimal essential medium with 10% unheated fetal calf serum, 2 mM glutamine, penicillin (250 units/ml), and streptomycin (250 μg/ml). For sarcoma virus focus assays, McCoy's 5A medium with antibiotics and 5% heated (56 C, 30 min) calf serum was used.

Viruses. Laboratory strains and recent field isolates of MuLV (5) were propagated in NIH or BALB/c ME cultures, depending on which type of culture was most sensitive. Strains which propagated equally well in the two types of culture were carried in NIH ME. Limiting dilution purification of several strains was carried out by two successive titrations in sensitive ME cells; each titration consisted of a 3-week test of serial 10-fold dilutions for complement-fixing (CF) antigen induction [5; the COMUL (complement fixation for murine leukemia) test] followed by a 3-week blind passage of each CF-negative culture harvest.

The nomenclature used for the field strains consists of the strain of mouse, an abbreviation for the type of tissue (L = leukemia; T = tumor; S = spleen; E = embryo), and an identifying number.

Focus-forming pseudotypes were obtained by the co-cultivation rescue procedure (7), by using the HT-1 line of hamster tumor cells induced by Moloney sarcoma virus (M-MSV) (10). The ME cell used for co-cultivation was the cell routinely used for passage of the leukemia virus.

Virus titrations. MuLV was quantitated by the fluorescent-antibody (FA) focus assay (W. P. Rowe, J. W. Hartley, and R. E. Wilsnack, *in preparation*) and by extinction dilution titrations with the COMUL procedure; MSV was titrated by focus assays (3) without added helper virus. Unless otherwise stated, the COMUL titers presented here represent the titration end points ($TCID_{50}$) obtained without blind passage.

Many of the titrations were done in cultures pre-

treated with diethylaminoethyl (DEAE)-dextran (16). Cultures were exposed to 25 μg of DEAE-dextran (Sigma Chemical Co.) per ml for 1 hr at 37 C, rinsed once or twice, fed with maintenance medium, and inoculated with virus. All comparisons of titers reported here are based on tests of identically treated cultures, i.e., both untreated or both DEAE-dextran-treated.

RESULTS

Relative susceptibility of NIH and BALB/c ME to various MuLV strains. Table 1 shows the results of comparative titrations of a number of virus strains in NIH and BALB/c ME cultures; in each case, the titer of virus in the more sensitive cell was $10^{4.5}$ to $10^{6.5}$ $TCID_{50}$ or FA focus-forming units per 0.1 ml. Three patterns were evident. Some strains, designated N-tropic, more easily initiated infection in NIH ME, others (B-tropic) grew preferentially in BALB/c ME, and others (NB-tropic) infected both with comparable efficiency. The same pattern was seen with COMUL, FA focus induction, and focus formation by M-MSV pseudotypes. The restriction was most marked in the COMUL assay, which requires secondary spread of virus in the culture for infection to be detected.

The strain of Friend virus used had been passaged in NIH Swiss mice followed by three passages in BALB/c ME and nine passages in NIH ME, including two limiting dilution passages in the latter cell. In contrast to the N-tropism of this passage line, a pseudotype prepared from a passage line of Friend virus which had been carried in BALB/c mice by J. B. Moloney gave equal focus induction titers in NIH ME and BALB ME.

It should be noted that pretreatment of the cultures with DEAE-dextran did not alter the resistance patterns; in both sensitive and resistant cultures, the treatment increased titers by 10- to 30-fold.

Tests of the M-MSV pseudotypes in vivo gave comparable susceptibility patterns (Table 2).

Evidence for genetic determination of host-range characteristics. Experiments were done to evaluate the role of nongenetic factors such as host-induced modifications or virus mixtures in determining the relative titers of the viruses in NIH and BALB/c ME cells.

The possibility was considered that the cell type in which the virus was grown determined the host range characteristics of the progeny. Restricted variants were passaged serially in the more resistant host cell, and the harvest at each passage was tested for ability to infect NIH and BALB/c ME cells. In no instance was a reversal of host range pattern seen. In general, after one to four passages in the resistant cell, the N/B ratio of the progeny was unchanged. If the cultures were not treated with DEAE-dextran, the strains were usually lost after two or three passages. With the enhancement provided by DEAE-dextran treatment, the strains could generally be maintained. The tropism of the original virus

TABLE 1. *Comparative sensitivity of NIH ME and BALB/c ME tissue cultures to infection with various murine leukemia viruses*

Strain	Subgroup	Ratio of titer in NIH ME to titer in BALB/c ME			Classification by tropism
		COMUL	FA FFU[a]	MSV pseudotype FFU	
Moloney[b]	FMR	1	1	3	Dual (NB)
Rauscher[b]	FMR	1/3		1/3	Dual (NB)
Friend[b]	FMR	2,000	300		N
Gross passage A[b]	Gross-AKR	10,000	160	160	N
AKR-L1[b]	Gross-AKR	1,000	160	200	N
C3H/Fg-E1	Gross-AKR		≥250		N
C3H/He-E1	Gross-AKR		400		N
BALB/c-S1[b]	Gross-AKR	5,000	60	30	N
BALB/c-T1[b]	Gross-AKR	<1/1,000	≤1/300	<1/2,500	B
BALB/c-S2N[b]	Gross-AKR	300	50		N
BALB/c-S2B[b]	Gross-AKR	<1/3,000	<1/800		B
WM1-B	ND[c]	3		1/1.3	Dual (NB)
C57BL-MCT1	ND		≤1/1300		B

[a] Focus-forming units.
[b] Virus pool tested was prepared with seed virus purified by two limiting dilution titration passages in NIH ME or BALB/c ME (BALB/c-T1 and BALB/c-S2B.)
[c] Not determined.

TABLE 2. *Pathogenicity of M-MSV pseudotypes of host-range variants for newborn NIH and BALB/c mice*

Pseudotype	Host range in tissue culture	Dilution tested	No. of mice with tumors/no. inoculated	
			NIH	BALB/c
M-MSV	NB	10^{-2}	16/16	12/12
		10^{-3}	2/5	9/16
		10^{-4}	0/14	2/12
M-MSV (Gross)	N	10^{-1}	12/16	0/8
		$10^{-1.6}$	8/15	0/16
M-MSV (C3H/He E1)	N	10^{-1}	17/17	0/17
M-MSV (BALB/c T1)	B	10^{-1}	0/15	12/12
		10^{-2}	0/15	6/6
M-MSV (BALB/c T2)	B	10^{-1}	0/15	13/13
		10^{-2}	0/15	11/15

was unchanged in most instances, although some strains showed a broadening of host range, with two B-tropic strains becoming essentially NB-tropic.

Further evidence of the lack of determination of tropism by the host cell used for passage is the recovery of a few strains of N-tropic virus from BALB/c ME inoculated with specimens from BALB/c mice. Also, pseudotypes rescued with ME cells infected with a restricted variant showed the host-range characteristics of that variant.

The failure to reverse tropism by passage of virus in the resistant cell suggested that the populations studied were not mixtures. However, it was important to evaluate the role of mixtures further, since all three host-range types showed some ability to propagate in both cell systems. This was particularly important with the isolates from BALB/c mice, in which B-tropic and N-tropic strains have been isolated from the same animal and even from the same tissue extract (e.g., strains BALB/c-S2N and S2B in Table 1).

Attempts to resolve possible mixtures by two limiting dilution passages in sensitive cells did not alter the host-range characteristics; i.e., Moloney virus remained NB-tropic and representative B- and N-tropic strains retained their limited degree of ability to infect the more resistant cell.

The results obtained with deliberate mixtures of N-tropic and B-tropic strains are shown in Table 3; these data show that the variants alone retained their host-range characteristics on passage in resistant cells and that the cultures inoculated with the mixtures preferentially grew the homologous variant. It thus appears that there is little, if any, helper effect of a B-tropic strain on the growth of an N-tropic strain in BALB/c cells and vice versa. Also, these results indicate that, if phenotypic mixing occurs, it is not an efficient process.

These findings indicate that host-induced modification is not a significant factor in determining the host-range characteristic, and that mixtures of N-tropic and B-tropic strains are not likely to be maintained as such in serial passage in either cell type. The data appear to be most compatible with the concept that the determination of host range is under the genetic control of the virus.

DISCUSSION

Genetic variation in host range for different species is common among animal viruses, and differences in susceptibility of mice of different inbred strains to various viruses, including leukemia viruses, is also well known. However, except for the avian leukosis viruses, it is unique to find naturally occurring host-range variants for different genetic lines of the natural host species. In the avian leukosis complex, host-range restriction is a function of serotype (17). This is not the case with the MuLV strains. Although most of the viruses of the FMR subgroup are dually tropic, possibly as a result of prolonged laboratory passage, the Gross-AKR antigenic subgroup contains both B-tropic and N-tropic strains (Table 1). However, it is possible that the N- and B-tropic strains constitute further antigenic subgroups within the Gross-AKR complex. No naturally occurring dually tropic virus has yet been found in the Gross-AKR subgroup, but preliminary studies indicate that NB-tropic

TABLE 3. *Virus production by mouse embryo cell cultures infected with mixtures of N-tropic and B-tropic murine leukemia viruses*

Inoculum ($TCID_{50}$)		Passed in[c]	CF antigen titer	Assay of infectivity (FA FFU[d]/0.1 ml) in 21-day harvest of		
BALB/c-Sl (N-tropic[a])	BALB/c-Tl (B-tropic[b])			NIH ME[e]	BALB/c ME[e]	N/B ratio
$10^{5.5}$	$10^{4.5}$	NIH ME	8	$10^{5.7}$	$10^{3.8}$	80
		BALB/c ME	8	$10^{4.3}$	$10^{5.9}$	1/40
$10^{3.5}$	$10^{4.5}$	NIH ME	16	$10^{5.8}$	$10^{3.7}$	130
		BALB/c ME	8	$10^{2.7}$	$10^{6.3}$	1/4,000
$10^{5.5}$	None	NIH ME	16	$10^{5.8}$	$10^{3.7}$	130
		BALB/c ME	16	$10^{4.8}$	$10^{3.2}$	40
None	$10^{4.5}$	NIH ME	<2	$<10^{2.5}$	$<10^{2.5}$	—[f]
		BALB/c ME	16	$10^{2.7}$	$10^{6.0}$	1/2,000

[a] N/B ratio was 100 as determined by COMUL test with blind passage.
[b] N/B ratio was 1/1,000 as determined by COMUL test with blind passage.
[c] Cultures were held 21 days, and the supernatant fluid was collected for assay. These cultures were not treated with DEAE-dextran.
[d] Focus-forming units.
[e] Assay plates were pretreated with DEAE-dextran.
[f] Ratio not calculable. In one experiment, this strain was successfully carried in serial passage in NIH ME, and the N/B ratio of the fourth passage harvest was < 1/450. In other experiments, this strain has tended to become NB-tropic when passed in NIH ME.

variants can be obtained by laboratory passage of B-tropic Gross-AKR type strains in NIH ME cells.

Since the selective pressure in the propagation of mixtures of N- and B-tropic strains was found to be in the direction of the homologous variant, it is unlikely that an established strain found to be NB-tropic represents a mixed population. However, a strain should not be considered dually tropic unless host range assays are performed with limiting dilution purified virus.

The degree of host-range restriction of MuLV in NIH and BALB/c ME cells is not as marked as with the avian leukosis viruses; this may be related to the similarly less marked difference in viral envelope antigens between serotypes of the MuLV group.

The limitation responsible for the low efficiency of infection in restricted cells is not known. It is noteworthy that the FA foci induced in resistant cells are as intensely stained, and often as large, as those in sensitive cells; this suggests that the restriction can be overcome by sufficient multiplicity.

The host-range restrictions described here constitute a major variable in the laboratory assay of MuLV. It seems probable that the failure to isolate leukemia virus from certain mouse strains such as NZB, in which C-type particles are abundant (9, 18), is due to as yet undefined host-range restrictions of the agent.

The host-range phenotype provides a useful marker in the study of the natural history of leukemia virus strains. It is noteworthy that the leukemia virus carried by a mouse strain may show relative restriction in cells of that strain, as exemplified by the recovery of many N-tropic strains from BALB/c mice. Further data on the occurrence of N-tropic and B-tropic viruses from BALB/c mice will be presented elsewhere.

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