

THE INFLUENCE OF THE PARTNER CELL ON THE PRODUCTION
OF L VIRUS AND THE EXPRESSION OF VIRAL SURFACE
ANTIGEN IN HYBRID CELLS*

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Increasing evidence indicates that the genetic constitution of the host has a complex interaction with virus infection in producing leukemia in the mouse. The genetic basis of leukemogenesis was established by the work of Furth et al. (1) and McDowell (2), who developed inbred high-leukemia strains. These authors also demonstrated that the incidence of leukemia in hybrids of high- and low-leukemia strains was proportional to the amount of genetic material derived from the high-leukemia parental strain (3, 4). At the same time it was clear that leukemogenesis was not inherited in a simple mendelian fashion.

Genetic control of susceptibility to leukemia viruses has been demonstrated in studies with the Gross (5) and Friend (6-9) murine leukemia viruses and with the avian leukemia-sarcoma complex (10-12). Recently, the naturally occurring murine leukemia viruses were found to fall into two categories in their behavior on mouse embryo cells of National Institutes of Health/Swiss (N) or BALB/c mice. N-tropic viruses initiate infection 100-1,000-fold more efficiently on N than B cells, while B-tropic viruses show the reciprocal pattern (13). The studies of Pincus et al. (14), using the XC-plaque test, indicated that the pattern of sensitivity to infection of cells from various inbred and hybrid mice was genetically determined, essentially by a single genetic locus. This locus has no effect on the growth of certain laboratory-passaged viruses, which infect both cell types equally well and are therefore designated NB tropic. The N-B locus was found to be identical with a major gene described for Friend virus susceptibility, *Fv-1* (15).

The question explored in the present paper is whether the *Fv-1* locus, which governs resistance to extraneous infection with leukemia viruses, also operates in somatic cell hybrids, in which one parent introduces the information for C-type virus production.

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Materials and Methods

Cells.—The A9 mouse fibroblast line was derived by Littlefield (16) from the L cell strain by selection for resistance to 8-azaguanine. The cells lack the enzyme hypoxanthine-guanine-phosphoribosyltransferase. This defect prevents their growth in the selective HAT medium (17). A9 cells have little ability to grow progressively in vivo. Inocula of 5×10^4 – 2×10^6 cells produced tumors in only 12% of X-irradiated, newborn, syngeneic C3H mice (18). One of these tumors was explanted, and a cell line was derived. When this line was inoculated into X-irradiated newborn syngeneic C3H or semiallogeneic C3H \times XF₁ mice (X designates a number of different allogeneic parents), 80–90% of the inoculated animals developed progressive tumors. The cell line was therefore designated A9HT (high take incidence). The A9HT line, like its A9 parent, is unable to grow in HAT medium (19).

Since the L cells were derived from a C3H mouse, both A9 and A9HT carried the *H-2^k* isoantigen complex. These cells have also been found to express two other surface antigens: the L antigen, present on the L cell strain and its derivatives (20), and a C-type virus-induced antigen, identical with, or cross-reacting with, the Friend, Moloney, Rauscher, and Graffi (FMRGi)¹ type-specific antigen. The C-type virus carried by L cells is morphologically indistinguishable from the murine leukemia viruses, but has no leukemogenic activity (21). Exposure of sensitive indicator cells (JLS-V9) to medium in which L cells have grown leads to the appearance of the FMRGi surface antigen in the indicator cells (22). Since we routinely use an anti-Moloney serum for its detection, this antigen will be referred to as Moloney-type cell surface antigen (MV-CSA). The JLS-V9 indicator cell system can be used to assay for infectious virus.

MSWBS is an ascites sarcoma, derived from the MSWBS tumor, originally induced by methylcholanthrene in an (A \times ASW)F₁ hybrid mouse (23). The MSWBS subline was isolated and subsequently passaged in the parental A.SW strain; it has lost the *H-2^a* isoantigen complex of the A strain, but has retained the *H-2^s* antigen of the A.SW strain. SEWA is an ascites sarcoma, induced by polyoma virus in an A.SW mouse (24). It carries the *H-2^s* complex and the polyoma-specific transplantation antigen (24). Both MSWBS and SEWA are specific for the A.SW strain and grow regularly from small inocula (23).

Ly(ASW) was the designation given to a suspension of normal lymph node cells from ASW mice; LyNIP were lymph node cells of an (A \times C57B1)F₁ hybrid mouse immunized with nitroiodophenyl acetic acid. Im.spl. were normal spleen cells of an (ACA \times C57B1)F₁ hybrid mouse immunized with sheep erythrocytes. Hybrid cells produced by the fusion of A9HT cells with host cells in vivo were also tested. What host cells were involved in the fusion in vivo is not known at present.

Cell Fusion and Selection of Hybrid Lines.—

(a) *In vitro fusion with the help of Sendai virus:* The cells were fused by means of inactivated Sendai virus, as described by Harris and Watkins (25). Hybrid cell lines were selected in HAT medium (16). MSWBS cells grow poorly in HAT; SEWA cells do not attach readily to glass or plastic surfaces; cells of the lymphoid series do not attach at all. These parental cells are therefore easily eliminated by changes of medium.

(b) *Isolation of tumor-host cell hybrids:* When tumors produced by the injection of A9HT cells were explanted and then grown in HAT medium, the great majority of the cells died within 1 wk; but after this time, clones of HAT-resistant cells appeared. Antigenic and chromosomal analyses showed that these HAT-resistant cells are indeed hybrids between the A9HT tumor cell and host cells (26). A9HT-host cell hybrids were derived from tumors produced in newborn, X-irradiated mice of the following genotypes: C3H \times C57BL (hybrids

¹ *Abbreviations used in this paper:* FMRGi, Friend, Moloney, Rauscher, and Graffi-type antigen; MV-CSA, Moloney-type cell surface antigen.

designated A9HT-H7), ACA \times C3H (A9HT-HC), C3H \times DBA/2 (A9HT-HD), and CBAT6T6 (A9HT-BT6). The time interval between initiation of selection in HAT medium and formation of a monolayer of HAT-resistant cells was 12–16 days. The cells used in the present experiments were between their first and fourth passages *in vitro*.

Chromosome Preparation.—Chromosome preparations of the ascites tumor cells and of *in vitro* cultures were made by the air-drying technique of Rothfels and Siminovitch (27). The cells were exposed to Colcemid (0.1 μ g/ml) for 2 h, treated with sodium citrate solution (0.9), and fixed with acetic acid-methanol (1:3). They were then spread on wet slides, air-dried, and stained with buffered Giemsa solution. 25 metaphases were examined in each sample.

Antisera.—Isoantisera were produced by immunizing groups of 10–13 adult mice with pooled cell suspension prepared from spleen, kidney, and liver and injected subcutaneously once every 2 wk for 14–24 wk. The mice were bled 7–10 days after the last injection. The sera were chosen to demonstrate the *H-2* antigens of one parent cell, with the exclusion of the *H-2* antigens derived from the other parent. The activity and specificity of all isoantisera were checked by cytotoxicity tests against lymph node cells of appropriate genotypes. None of the isoantisera used had detectable anti-Moloney activity when tested against MLV-infected JLS-V9 cells (BALB/c origin, *H-2^d*) or against the Moloney virus-induced YBA lymphoma (CBA origin, *H-2^a*).

Anti-Moloney serum was collected from (ABY \times DBA/2) F_1 hybrid mice 90–150 days after the last inoculation of cells of a syngeneic Moloney lymphoma (YDYA). Three inoculations, each of 5×10^6 cells, given 6,000 rad of X irradiation, were made at weekly intervals. This serum was active to a titer of 1:32 in cytotoxicity tests against YAC target cells (Moloney lymphoma of strain A origin, *H-2^a*).

A typing serum for the L-type surface antigen was collected from (C3H \times DBA/2) F_1 hybrid mice immunized with A9 cells subcutaneously at fortnightly intervals for 21 wk. The mice were bled 7–10 days after each injection, beginning with the third, and the sera were pooled. The pooled serum was active to a titer of 1:3,000 in indirect membrane immunofluorescence tests against A9 target cells. The serum did not react with Moloney virus-infected JLS-V9 cells or with Moloney lymphoma cells (YAC), indicating the absence of antibodies against histocompatibility antigens present in the A or BALB/c strain, or against the Moloney virus-controlled surface antigen (MV-CSA). All sera were stored at -20°C .

Indirect Membrane Immunofluorescence Test.—This test was done on viable cells as previously described (28). Cells in confluent monolayer cultures were suspended mechanically by means of a rubber policeman. Only suspensions with at least 80% living cells were used. Hanks' balanced saline solution containing 1% gelatin was used to wash the cells and for serial dilution of antisera. All sera were tested undiluted and at dilutions of 3, 10, 30, 100, 300, 1,000, and 3,000. The fluorescein-labeled goat antimouse gamma globulin was obtained from Hyland Laboratories, Div., Travenol Inc. (Los Angeles, Calif.) and was used at a dilution of 1:20. Approximately 100 cells were classified by criteria previously described (28). A fluorescence index was calculated by subtracting the percentage of negative cells in the test sample from the corresponding value in a sample exposed to normal mouse serum and dividing the difference by the latter figure. As a rule, 80–90% of the cells were negative in the control.

Adsorption Tests.—To measure the concentration of antigens on the surface of the cell lines, quantitative adsorption tests were done as described previously (29). 0.03 ml of the antiserum was diluted in phosphate buffer containing 1% gelatin to give an 80–90% killing of sensitive target cells. This diluted antiserum was absorbed with varying numbers of test cells, ranging from 6×10^4 to 4×10^6 . The cells were washed twice with buffer before test and were added to the antiserum in an equal volume of buffer. The preparation was incubated for 30 min at 37°C and then allowed to stand for 30 min at 4°C . The supernatant was then cleared by two cycles of centrifugation at 5,000 rpm and stored frozen at -20°C . Cytotoxic activity of antisera was tested as described previously (30), except that rabbit serum was the

source of complement when anti-Moloney serum was used. This change was necessary to increase the sensitivity of the test. The target cells were lymph node cells from appropriate donors for *H-2* antisera and the Moloney lymphoma YAC for anti-Moloney serum.

In order to compare the adsorptive capacity of the various cells for the single pool of anti-serum that was used throughout, the ratio of bound to free antibody, $(100 - P)/P$, was calculated by the method of Reif and plotted on log-log paper against the ratio g of the number of cells to the volume of antiserum used in the adsorption (31). A regression line was calculated by the method of least squares and the value of G ($= g$ when $P = 50\%$) was interpolated. The reciprocal $1/G$ was used as a single numerical estimate for the adsorptive capacity of the different cell types.

Infectivity Assay.— 2.5×10^5 cells were seeded in milk dilution bottles in Eagle's minimum essential medium containing 10% fetal calf serum. The medium was collected after 24 h of growth and spun at 1,500 g for 10 min. Ascites tumor cells grown *in vivo* were tested for release of infectious virus as described previously (22). 1 ml of undiluted culture fluid or fluid diluted 1 in 10, 10^2 , or 10^3 were used to infect JLS-V9 cells or mouse embryo fibroblasts.

JLS-V9 test: We have previously shown (22) that exposure of JLS-V9 cells to medium from L cell cultures results in the induction of Moloney virus-controlled surface antigen (MV-CSA). This observation formed the basis of an assay for virus release by the various hybrid lines.

JLS-V9 cells were seeded into bottles 5 h before infection and infected as described previously (32). The virus-infected and control cultures were subcultured at a 1:10 reduction twice a week and tested for MV-CSA-specific membrane fluorescence each week. Positive tests were repeated at least once. Negative cultures were carried for 5 wk after infection and then discarded.

XC-plaque test: The plaque assay technique for murine leukemia viruses was developed by Rowe et al. (33). It is based on the finding that cocultivation of MLV-infected cells with XC cells, a rat tumor cell originally induced by the Prague strain of Rous sarcoma virus (34), results in giant cell formation of XC cells. Secondary cultures of mouse embryo fibroblasts were seeded in 50-mm plastic Petri dishes at a density of 2×10^5 cells/dish. The cultures were grown on Eagle's minimum essential medium containing 10% unheated fetal calf serum and 5.12 mg of glutamine/ml. 24 h after seeding, the cells were treated with 100 $\mu\text{g/ml}$ of DEAE-dextrane for 1 h, rinsed once with phosphate-buffered saline, and then infected. 1 ml of undiluted or diluted virus was allowed to adsorb onto the cells for 60 min, and the cells were then rinsed with medium. Culture fluids were changed every 2-3 days. The cultures were exposed to ultraviolet light for 30 s ($60 \text{ erg/mm}^2 \text{ per s}$) 7-10 days after inoculation, in order to detach the embryo fibroblasts. Immediately after irradiation, 10^6 XC cells were added to each Petri dish. The dishes were fixed in methanol 3-4 days later (after one further change of medium) and were stained with hematoxylin.

RESULTS

Identification of Hybrid Cell Lines.—The chromosomal constitution of parental and hybrid cells is shown in Table I. The karyotype of the A9HT line was found to be similar to that of the A9 line, but with a slightly reduced chromosome number. The modal chromosome number of A9HT was about 53, compared with about 57 for A9. A9 and A9HT both had between 20 and 30 bi-armed chromosomes. A detailed comparison of the chromosomes of A9 and A9HT made by the quinacrine fluorescence technique is given in the paper by Allderdice et al. (35). 90% of SEWA tumor cells contain a marker chromosome with a secondary constriction; this could be identified in approximately 50% of the hybrid metaphases. The MSWBS (23) tumor cells contain 10 bi-

TABLE I
Chromosome Constitution of Parental and Hybrid Cells

Cell line	Total number of chromosomes*		Number of biarmed chromosomes‡	
	Range	Mode	Range	Mode
Parents				
A9	53-58	56	24-28	26
A9HT	50-53	53	25-27	25
MSWBS	28-30	29	8-11	10
SEWA	42-44	43	0-1	1
Normal mouse cells	40	40	0	0
Hybrids				
MSWBS-A9	57-78	68	22-32	26
SEWA-A9	78-96	(90)§	20-26	22
Ly(ASW)-A9	72-89	75	27-38	32
LyNIP-A9	61-85	69	19-25	20
imm.spl.-A9	70-96	74	17-25	23, 25
A9HT-BT6	89-97	92	22-27	23
A9HT-HD	69-82	—	19-31	—
A9HT-H7	74-93	—	20-23	24, 25
A9HT-HC	79-94	92	19-28	25

* 20-55 metaphases were counted for each cell line.

‡ The A9-derived biarmed chromosomes were identified in all hybrids. All A9HT-BT6 hybrids contained the T6T6 marker from the CBA mouse. Approximately 50% of the SEWA-A9 hybrids contained one distinct SEWA marker.

§ Figure in parentheses denotes a weak mean.

armed chromosomes; but since they are indistinguishable from the metacentric A9 chromosomes in the hybrid cell, they could not serve as markers. The MSWBS-A9 hybrids and those hybrid cells in which one partner was a normal mouse cell were identified by the total chromosome number, the number of biarmed chromosomes, and specific surface antigen markers. The hybrid nature of the A9HT-BT6 hybrid cell was confirmed by the presence of biarmed chromosomes from the A9HT parent cell and the T6T6 markers from the host animal.

The MSWBS-A9 hybrid line had lost both biarmed (—10) and acrocentric (—17) chromosomes. Its hybrid nature was nonetheless confirmed by the presence of the $H-2^s$ and $H-2^k$ isoantigens derived from the MSWBS and A9 cells. (Table II and Fig. 1). The chromosome constitution of the SEWA-A9 cells varied over a wide range. In part of the population, the chromosome numbers approximated to the sum of the parental chromosomes, although the modal number was below that expected for the sum of the two parent cells (—6). However, antigen markers from both parental cells, the $H-2^s$ (derived from SEWA) and $H-2^k$ and L antigens (derived from A9) confirmed the hybrid nature of the cell line.

TABLE II
Surface Antigens in Parental and Hybrid Cell Lines*

Cell lines	Cell genotype	Typing serum										L type	
		H-2 ^k		H-2 ^s		H-2 ^d		H-2 ^b		H-2 ^f			
		ASW	(C57BL X DBA/2)F ₁ Anti-C3H	(C57BL X ACA)F ₁	C3H Anti-ASW	(C57BL X C3H)F ₁ Anti-DBA/2	(C57BL X C3H)F ₁ Anti-C57BL	A/Sr Anti-C57BL	(ACA X C3H)F ₁	A/Sr Anti-ACA			
A9	H-2 ^k	MIF	MHA	MIF	MHA	MIF	MHA	MIF	MHA	MIF	MHA	MIF	MHA
MSWBS	H-2 ^s	30§	100	100	10	10	Neg	Neg	Neg	Neg	Neg	Neg	100
MSWBS-A9	H-2 ^s , H-2 ^k	Neg	10		Undil.	100	10	Neg	Neg	Neg	Neg	Neg	1,000
SEWA	H-2 ^s	Neg			30								Neg
SEWA-A9	H-2 ^s , H-2 ^k	Undil.	100		10	100							Neg
Ly(ASW)-A9	H-2 ^s , H-2 ^k	10	300		30	100							100
Ly(NIP)-A9	H-2 ^s X H-2 ^k , H-2 ^k						Neg	Undil.	Neg	Neg			100
imm.spl.-A9	H-2 ^f X H-2 ^b , H-2 ^k												100
													30

* The data in this table are based on three to four independent tests. MIF, membrane immunofluorescence; MHA, mixed hemadsorption; Neg, negative; Undil., undiluted.
 † The antisera were titrated against appropriate lymph node cells in a cytotoxicity test. The titer for 50% lysis is indicated.
 § Figures denote the reciprocal of serum dilution at which 50% of the cells reacted.

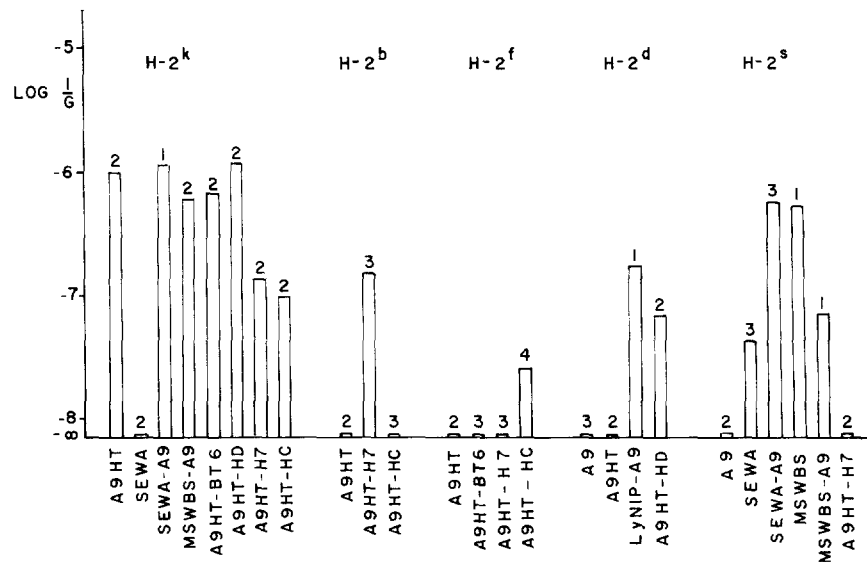


FIG. 1. Adsorptive capacity of parental and hybrid lines for ACA \times C3H anti-C57BL ($H-2^b$), ACA \times C57BL anti-C3H ($H-2^k$), A anti-ACA ($H-2^f$), ACA \times C3H anti-DBA ($H-2^d$), and ACA \times C3H anti-ASW ($H-2^s$) serum. $1/G$ indicates the amount of antibody bound per cell when 50% of the cytotoxic activity of the antiserum had been bound. The numbers at the top of columns denote number of adsorption tests.

In the Ly(ASW)-A9 cells, both parental $H-2$ complexes and the L-type antigen were also expressed. It is interesting to note that the number of biarmed chromosomes in this hybrid has increased, as reflected by the range of the biarmed chromosomes (A9, 24-28; Ly(ASW)-A9, 27-38). LyNIP-A9 expressed the $H-2^d$ antigen (demonstrated in mixed hemadsorption and quantitative adsorption tests) derived from the A \times C57BL ($H-2^a-H-2^b$) parent cell, but seemed to lack $H-2^b$. Similarly, the im.spl.-A9 hybrid expressed very weakly, if at all, the $H-2^b$ antigen (reaction obtained only in mixed hemadsorption with undiluted antiserum), while the $H-2^f$ isoantigenic marker derived from the C57BL \times ACA spleen cell and the $H-2^k$ and L-type antigens of the A9 cell were present.

The antigen markers of A9HT-host cell hybrids were demonstrated by quantitative adsorption of specific antisera. A9HT-H7 cells, isolated on selective HAT medium from an A9HT tumor grown in a C3H \times C57BL F_1 hybrid mouse, absorbed $H-2^b$ antibodies in addition to $H-2^k$; A9HT-HC cells absorbed $H-2^f$ antibodies (derived from a tumor growing in ACA \times C3H F_1 mouse) and A9HT-HD cells (C3H \times DBA) absorbed $H-2^d$ antibodies.

Expression of Moloney-Type Cell Surface Antigen (MV-CSA) in Hybrid Cells.—Fig. 2 shows that this antigen was absent from the parental MSWBS and SEWA cells, since they did not react with anti-Moloney serum either in

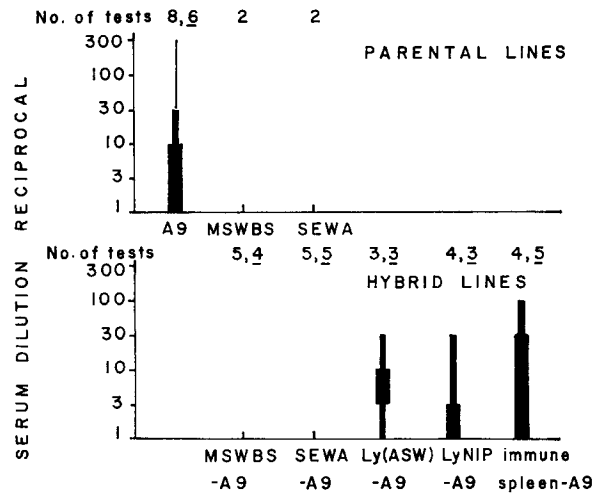


FIG. 2. Reactivity of cells in membrane immunofluorescence and mixed hemadsorption tests. The thickness of the column corresponds to the fluorescence index, at the stated serum dilution (ordinate). Fluorescence index, ■, 0.5–0.75; ▨, 0.25–0.5; |, reactivity in mixed hemadsorption only or when reactivity in mixed hemadsorption exceeded reactivity in membrane immunofluorescence. The number of repeated tests is shown at the top of each column; the number of mixed hemadsorption tests is underlined.

membrane immunofluorescence or in mixed hemadsorption. Furthermore, normal mouse cells lack this antigen, as a rule. Thus in hybrid cells this antigen, when present, was introduced by the A9 or A9HT partner. The Moloney-type antigen was not demonstrable in the MSWBS-A9 and SEWA-A9 hybrid cells, while the Ly(ASW)-A9, LyNIP-A9, and im. spl.-A9 cells expressed it about as strongly as the A9 cell, judged by the similar reactivity of the hybrid cells with anti-Moloney serum.

Fig. 3 illustrates the adsorptive capacities of A9 and A9HT parental lines and A9HT-H7, A9HT-HC, A9HT-HD, and A9HT-BT6 hybrid cells with respect to an anti-Moloney serum. Residual cytotoxic activity was tested against the Moloney lymphoma YAC. While the two parental lines and the A9HT-BT6 and A9HT-HD hybrid had a similar adsorptive capacity, indicating closely similar Moloney-specific antigen concentration on their surfaces, A9HT-H7 and A9HT-HC hybrid cells showed a higher amount of this antigen. Lymph node cells from normal CBA mice or secondary mouse embryo fibroblast cultures of C57BL origin did not remove anti-Moloney activity from the test serum.

Virus Production of Parental and Hybrid Cells Detected by the JLS-V9 Test.—The L cell derivatives A9 and A9HT lines release large amounts of infectious virus, since a 100- or 1,000-fold dilution of the culture medium was still infectious (Table III). MSWBS and SEWA tumors did not release infectious virus,

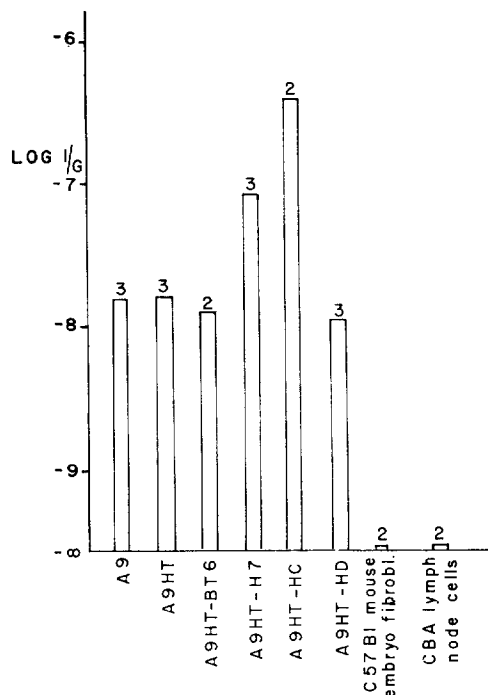


FIG. 3. Adsorptive capacity of A9 and A9HT parental lines and A9HT-BT6 and A9HT-H7 hybrid lines of anti-Moloney serum. For significance of 1/G value, see legend for Fig. 1.

nor did their hybrids with A9 cells. Similarly, no infectious virus could be recovered from the medium of Ly(ASW)-A9, LyNIP-A9, im.spl.-A9, and A9HT-H7 and A9HT-HC hybrid cultures. On the other hand, undiluted culture fluid from A9HT-BT6 or fluid diluted 1 in 100 and undiluted fluid from A9HT-HD hybrid cells contained virus infectious for JLS-V9 cells.

Characterization of L Cell Virus for N or B Tropism.—Secondary mouse embryo fibroblast cultures of C3H (N type) and C57BL (B type) origin were infected with the culture fluid from A9, A9HT, or YAC lymphoma cell cultures. 7–10 days later, the fibroblast monolayers were detached by UV-irradiation and overlaid with XC cells. The ability of XC cells to undergo giant cell formation on contact with monolayers infected with mouse leukemia virus was the measure of virus infection.

The results are shown in Table IV. The culture fluid from the Moloney lymphoma YAC infected both C3H and C57BL mouse embryo fibroblasts successfully and showed plaque formation on XC cells when overlaid with this indicator system. On the other hand, the L cell virus, derived from four different sublines of L cells, infected only C3H embryo fibroblasts, which are N

TABLE III
Virus Release in A9 and A9HT Cells and in Hybrid Cell Lines

Number of infectivity tests	Culture medium		
	Source	Undiluted	Diluted 10 ⁻²
Parent Cells			
4	A9	+++	+++
2	A9HT { tumor 1	+++	++
	tumor 2		++
	tumor 3		+
2	MSWBS	-	-
3	SEWA	-	-
Hybrids			
2	MSWBS-A9	-	-
4	SEWA-A9	-	-
3	Ly(ASW)-A9	-	-
2	LyNIP-A9	-	-
3	imm.spleen-A9	-	-
2	A9HT-BT6	++	+
2	A9HT-HD	++	-
2	A9HT-H7	-	-
2	A9HT-HC	-	-

Virus release was assessed by the ability of culture media to induce the Moloney-type antigen in JLS-V9 cells. The time necessary for the appearance of specific membrane immunofluorescence was a function of virus dilution. If a fluorescence index of 0.50 was produced within 7 days, the result was scored + + + +; within 7-14 days, + + +; within 14-21 days, + +; within 21-28 days, +. When the fluorescence index did not exceed 0.25 during the 5 wk of observation, the result was indicated by -.

type. While overlay of the infected fibroblasts with XC cells did not result in macroscopically visible plaques (14), solitary giant cell formation was detectable by microscopic examinations. These giant cells contained up to 2-20 nuclei and constituted only a small fraction of the cell population (2-10% at most). The XC monolayer on the infected C57BL fibroblasts showed no more giant cell formation than uninfected cultures. The L cell virus thus appears to infect C3H fibroblasts (N type), but not C57BL fibroblasts (B type); and we therefore conclude that it is N tropic.

DISCUSSION

When a Moloney lymphoma subline with a low level of expression of Moloney virus surface antigen (MV-CSA) and a low level of virus release was fused with A9 cells, the resulting hybrid cells expressed the antigen fully, but did not produce infectious virus. This result led us to conclude that the two properties are not coupled (37). In the present study, further evidence is obtained for

this view. We have tried to deduce some rules that may govern the expression of the two viral functions.

When the A9 cell (virus positive, antigen positive, V+A+) is fused to V-A- cells, three types of hybrids result (Fig. 4). They may be similar to either the V-A- or V+A+ parent, or they may not release infectious virus but may express the virus-controlled antigen (V-A+).

This complete (V-A-) or partial (V-A+) suppression of viral functions may be explained by the N-tropic character of the L cell virus. On the basis of XC giant cell formation on C3H (N type), but not C57BL (B type), monolayers, the L cell virus may be considered an N-tropic virus. This is in agree-

TABLE IV
Detection of L Cell Virus in the XC Test

Virus			Mouse embryo fibroblasts			
Designation	Source*	Dilution	Plaque formation		Giant cell formation‡	
			C3H N-type	C57BL B-type	C3H N-type	C57BL B-type
L cell virus	A9	Undil.	-	-	+	-
	A9HT { tumor 1 tumor 2 tumor 3	10 ⁻²	-	-	-	-
		Undil.	-	-	+	-
		Undil.	-	-	+	-
Moloney leukemia virus	YAC	Undil.	Confl.	Confl.	+++++	+++++
		10 ⁻¹	83, 78	46, 33	++++	++++
		10 ⁻²	7, 3	14, 6	+++	+++
		10 ⁻³	0, 0	0, 0	+	+

* Culture fluid (minimal essential medium with 10% fetal calf serum) harvested 18-24 h after seeding of culture lines or explantation of the YAC lymphoma.

‡ Evaluated by the method of Johnson et al. (36). +++++, essentially all XC cells involved in the fusion, with numerous, extremely large, bizarre cells apparent; +++, fusion involves roughly one-half of the cells, 10-30 nuclei per cell; +, small number of multinucleated cells containing a few nuclei (2-10) per cell. Read at × 100 magnification.

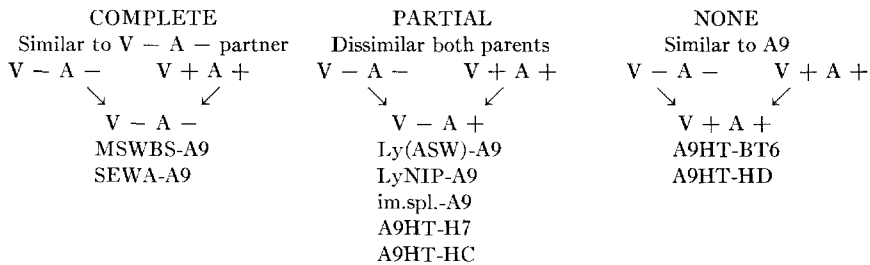


FIG. 4. Suppression of virus-related traits.

ment with the results of J. W. Hartley and W. P. Rowe (personal communication), who found that fluids from L cells induce a fluorescent antigen in N-type cells, but no plaques. In the hybrid lines the A9 cells were partnered with ASW components (MSWBS and SEWA tumors and normal lymph node cells), (A \times C57BL) F_1 lymph node cells, and (ACA \times C57BL) F_1 spleen cells. Cells of the C57BL and A mouse strains were shown to be B type (14). Since no gene closely associated with the *H-2* locus in linkage group IX plays a significant role in determining susceptibility to mouse C-type virus infection in vitro, the A.SW and A.CA strains, congenic with A, are probably B type. In two of the A9HT hybrid lines, the partner cell was an N \times B F_1 cell, (C3H \times C57BL) F_1 in the A9HT-H7 hybrid and (ACA \times C3H) F_1 in the A9HT-HC hybrid. Cells of CBA and DBA/2 mice were shown to belong to the N-type group (14).

A summary of the characteristics of the hybrid lines appears in Table V. The three V-A- hybrid lines were of N- and B-type combinations (MSWBS-A9 and SEWA-A9). Five lines showing no release of infectious virus but with expression of MV-CSA (V-A+) were also hybrids, in which a B-type cell or an N \times B F_1 -type cell was the partner. The hybrid lines selected from the A9HT tumors are of special interest, since the partner cell derived from the host probably belongs to the same physiological cell type in each case. The demonstrated difference in virus production may therefore depend entirely on the N or B type of the mouse strain. Since resistance to infection appears to be dominant, fusion with a B-type cell may have suppressed infectious virus release. One would then expect that fusion with an N-type cell would not alter virus release in the hybrid, and this is found to be the case in A9HT-BT6 and A9HT-HD hybrids.

TABLE V
Summary of Virus-Related Functions

Cell line	Virus-related functions		Character of partner cell
	Membrane antigen	Production of infectious virus	
A9	+	+	N
A9HT	+	+	N
MSWBS-A9	-	-	B
SEWA-A9	-	-	B
Ly(ASW)-A9	+	-	B
LyNIP-A9	+	-	B \times B
imm.spl.-A9	+	-	B \times B
A9HT-H7	+	-	N \times B
A9HT-HC	+	-	N \times B
A9HT-BT6	+	+	N \times N
A9HT-HD	+	+	N \times N

It is interesting to note that quantitative comparisons of the amount of MV-CSA present showed differences among the various hybrid lines. Whereas the virus-producing cell lines had closely similar adsorptive capacities, ranging from 3×10^{-7} to 4.5×10^{-7} , the two nonproducing hybrid lines showed a 1-log or higher increase in the amount of the surface antigen (2.3×10^{-6} for A9HT-H7 and 1.1×10^{-5} for A9HT-HC).

The L cell virus is readily detected by its ability to induce MV-CSA in JLS-V9 cells (22). The JLS-V9 cell line is derived from BALB/c bone marrow and is thus a B-type cell line. To reconcile the two facts, i.e. N tropism of the L virus with respect to the fibroblast but infectibility of the BALB/c JLS-V9 cell, one would have to assume that this cell line is more sensitive to infection than the primary embryo culture. This higher sensitivity of the JLS-V9 cell line may be explained by the properties of the system. First, the difference in infectivity between N- and B-tropic viruses is not absolute, but quantitative. Secondly, there are variations among different mouse strains belonging to the same group of tropism, e.g., the sensitivity of C57BL embryo cells to virus infection is 30% lower compared with BALB/c embryo cells; they are both B type. Finally, tissue culture lines are, as a rule, more sensitive to virus infection than secondary embryo fibroblasts.

In conclusion, it appears that the major genetic locus affecting resistance to infection with murine leukemia viruses, the *Fv-1* locus, also regulates infectious virus production in somatic cell hybrids. When a virus-producing cell (A9, N type) is fused with different partners, virus production may be suppressed in those hybrids in which the partner cell has a genotype that determines low infectibility with that particular virus (B type). The same genetic locus did not seem to govern the expression of all virus-related functions, for the virus-determined membrane antigen was demonstrated in many hybrids in which production of infectious virus was suppressed.

SUMMARY

The C-type particles produced by the A9 and A9HT sublines of mouse L cells were shown to infect C3H (N type), but not C57BL (B type), mouse embryo fibroblasts. Infection was indicated by distinct single giant cell formation in the XC monolayer used to overlay the mouse embryo fibroblasts. On the basis of these results it was concluded that the L cell virus is N tropic.

A9 and A9HT cells were fused to various mouse cells derived from tumors and normal tissues. The ability to produce the Moloney-type surface antigen and to release infectious virus was introduced by the A9 component into the hybrid cell. Virus production, measured by antigen induction on JLS-V9 cells, was suppressed in those hybrids in which the partner cell had a genotype determining low infectibility with that particular virus (B-type cell). It thus appears that the major genetic locus affecting resistance to infection with leukemia viruses, the *Fv-1* locus, regulates infectious virus production in somatic

cell hybrids also. The same genetic locus did not seem to govern the expression of all virus-related functions, for the virus-determined membrane antigen was demonstrated in many of the N × B-type hybrids in which production of infectious virus was suppressed.

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