Evidence for *H*-2-Linked Control of Retrovirus Production in Friend Virus-Induced Tumor Cell Lines

JOHN H. WOLFE† AND KENNETH J. BLANK*

Department of Pathology and Laboratory Medicine, Division of Research Immunology, University of Pennsylvania
School of Medicine, Philadelphia, Pennsylvania 19104

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In Friend leukemia virus-induced tumor cell lines derived from mice congenic with respect to the *H*-2 complex, most cell lines expressing the *H*-2^k haplotype continuously produced infectious exogenous virus in culture, whereas most cell lines expressing the *H*-2^b or *H*-2^d haplotype stopped producing virus during in vitro passage. This apparent *H*-2-linked control of virus production did not appear to be the result of alteration of the provirus or resistance to superinfection. The implications of this finding with respect to virus-induced leukemogenesis are discussed.

In the mouse, several genes influence susceptibility and resistance to retrovirus-induced leukemogenesis (33, 46, 54, 58). For most retroviruses, one or more of these genes is linked to the major histocompatibility complex, *H*-2, and is associated with the *H*-2K/*H*-2I or *H*-2D region or both (33, 54, 46). The mechanism for this *H*-2-associated control of susceptibility and resistance to virus-induced leukemogenesis remains poorly understood. In vivo, both immune responses to viral antigens (5, 7, 23, 24, 34, 40-42) and levels of virus replication (11, 21, 32, 38, 59, 64) have been shown to be influenced by genes linked to *H*-2. In these experiments, however, it is unclear if the observed control of virus production was a direct effect on some step in the life cycle of the virus or a secondary event perhaps related to the elimination of virus, virus-infected cells, or both, mediated by an immune response controlled by an *H*-2-linked immune-response gene.

To examine the possible direct effects of *H*-2-linked genes on virus expression in the absence of any interaction with the host (e.g., an immune response), we have established a series of cell lines from tumors induced by Friend virus (FV) in BALB mice congenic with respect to the *H*-2 complex. A few cell lines derived from tumors induced in mice from strains other than BALB/c were also examined. Previous studies on a small number of such cell lines suggested that an *H*-2-linked gene(s) might exert intracellular control over virus expression (18, 62, 63).

We now report that most cultured tumor cell lines derived from mice expressing the *H*-2^k haplotype continued to produce infectious, oncogenic virus of the type used for the initial infection in vivo, whereas most of the cell lines derived from mice expressing the *H*-2^b and *H*-2^d haplotypes stopped producing infectious virus in culture. The few tumor cell lines derived from mice with non-BALB backgrounds exhibited the same pattern of virus production with respect to *H*-2. Thus, these studies suggest that a gene(s) linked to *H*-2 may control the production of exogenous retroviruses by these cultured tumor cell lines. This control appeared to be exerted at some point in the life cycle of the virus after insertion of the provirus into the mouse genome, because nonproducer tumor cells continued to express virus anti-

gens, and the production of infectious exogenous virus by nonproducer cells was induced by treatment with iododeoxyuridine (IUdR).

MATERIALS AND METHODS

Mice. C57BL/10Lil (B10), BALB/c-*H*-2^k/Lil (BALB.K), and BALB/c-*H*-2^b/Lil (BALB.B) mice were bred from pairs obtained from F. Lilly, Albert Einstein College of Medicine, Bronx, N.Y. C57BL/10-*H*-2^k (B10.K) mice were bred from pairs obtained from D. Schreffler, Washington University, St. Louis, Mo. (BALB/c × NFS)F₁ mice were obtained from breeding the parent strains obtained from P. O'Donnell and A. Oliff, respectively, Sloan-Kettering Institute, New York, N.Y. BALB/c and (C57BL/6 × DBA/2)F₁ mice were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Viruses. FV (19) used in these studies was also originally obtained from F. Lilly as an N→NB-tropic virus. Virus stocks were maintained in this laboratory by serial passage in BALB mice inoculated as neonates. Virus stocks were prepared from 10% homogenates in Hanks balanced salt solution (HBSS) of the greatly enlarged thymus, spleen, and lymph nodes from a susceptible mouse inoculated with a high dose of virus. These homogenates were kept continuously at 0 to 4°C and were clarified by centrifugation for 15 min at 2,400 × *g*. The virus supernatants were stored in 2-ml ampoules at -70°C.

Tumor cell lines. All cell lines were produced and passaged in this laboratory, except for the Hfl/b-tc (clone L) and Hfl/d-tc tumor cell lines (17) which were originally obtained from H. Freedman, Downstate Medical School, Brooklyn, N.Y., and maintained in this laboratory for several years. The tumor cell lines used in this study were established by the following method. Mice were inoculated with FV intraperitoneally as adults. FV produces an erythroid leukemia characterized by splenomegaly in 2 to 3 weeks. These leukemic spleens were removed, minced into single cell suspensions, and seeded in tissue culture flasks in medium containing 10% fetal calf serum and 10⁻⁵ M 2-β-mercaptoethanol. Periodically, a portion of the medium was withdrawn, and fresh medium was added.

Detection of viruses. Ecotropic murine leukemia virus was detected in the XC plaque assay described previously (50). SC-1 (5 × 10⁴) cells (26) were seeded in six-well (9.6 cm² per well) tissue culture plates and incubated at 37°C in 5% CO₂ overnight. These cells were incubated for 45 min in 5 μg of

* Corresponding author.

† Present address: Laboratory of Veterinary Oncology, Sloan-Kettering Institute for Cancer Research, New York, NY 10021.

DEAE-dextran per ml and washed, and the sample to be tested, either culture supernatant or lethally irradiated (5,000 R) tumor cells (infectious center assay), was added. Supernatants were collected by resuspending 100% viable tumor cells in fresh medium at 10^6 cells per ml, incubating this culture for 18 h at 37°C, removing the cells by centrifugation, and storing the supernatant at -70°C. These supernatants or cells were removed 2 to 4 h later, and fresh medium with 10% fetal calf serum was added. These cultures were fed as needed. When the SC-1 cells were confluent, the medium was removed, the cells were exposed to 25 s of UV radiation, 10^6 XC cells in 2 ml of medium were added, and these cultures were incubated at 37°C in 5% CO₂ for 3 days. Supernatants were removed, cells were washed, and the cell monolayer was either stained and fixed for 5 min in a solution of 3 g of methylene blue stain, 500 ml of methanol, and 100 ml of carbolfuchsin or fixed in methanol for 5 min, dried, and stained with cresyl violet. These stained monolayers were examined for plaques. SC-1 and XC cells were a gift of J. Hartley, National Cancer Institute, Bethesda, Md.

This method was also used to determine *Fv-1* tropism by employing embryo fibroblasts derived from BALB (*Fv-1^b*), DBA/2 (*Fv-1ⁿ*), C3H/HeJ (*Fv-1ⁿ*), (BALB/c × NFS)F₁ (*Fv-1^{n/b}*), or (C57BL/6 × DBA/2)F₁ (*Fv-1^{n/b}*) mice or NIH-3T3 (*Fv-1ⁿ*) cells as target cells. NIH-3T3 cells were a gift of W. Hardy, Jr., Memorial Sloan-Kettering Institute. Embryo fibroblast lines were established by surgically removing embryos from female mice at 12 to 16 days of gestation, making single cell suspensions from them, and transferring the cells to tissue culture flasks in medium supplemented with 10% fetal calf serum. These adherent cells were passed as necessary.

FV activity was also measured *in vivo* by assaying for the formation of spleen foci and splenomegaly (2). Culture supernatants (0.5 ml) from 10^6 cells per ml growing at the logarithmic phase for 18 h and passed through a 20-μm-pore filter were injected intravenously into the tails of (C57BL/6 × DBA/2)F₁ (*Fv-1^{n/b}*) mice. Beginning 9 days after infection, mice were palpated for splenomegaly. Mice were sacrificed when splenomegaly developed or at day 14. Spleens were removed and fixed in Bouin solution, and fixed spleens were compared by size with uninfected spleens from the same strain of mouse. In most cases, spleens were either greatly enlarged (10 to 30 times larger) with confluent foci or indistinguishable from uninfected organs. Spleen foci were enumerated 9 days after intravenous injection of culture supernatants.

Supernatants from virus cultures were tested for the presence of reverse transcriptase as an indication of the presence of virus particles by the oligo(dT) priming method (22). Culture supernatant (25 μl) to be tested was mixed with 50 μl of 50 mM Tris hydrochloride (pH 8.0), 20 mM 2-β-mercaptoethanol, 5 mM MgCl₂, 60 mM NaCl, 0.05% Nonidet P-40, 5 μg of oligo(dT) per ml (Sigma Chemical Co., St. Louis, Mo.), 10 μg of poly(A) per ml (Sigma), and 20 μCi of [³²P]dTTP per ml (Amersham Corp., Arlington Heights, Ill.). This mixture was incubated 2 h at 37°C. A 50-μl sample was removed and blotted onto DEAE paper (Whatman DE-81; Whatman, Inc., Clifton, N.J.). The filters were washed three times for 30 min in 2 × 0.3 M NaCl-0.03 M sodium citrate and once in 95% ethanol, dried, and exposed to X-ray film for 24 to 60 h.

Detection of cell surface class I antigens. Cells were tested for cell surface expression of H-2 class I antigens by fluorescence-activated flow cytometry. Antibodies in solution were clarified by centrifugation in a Beckman Airfuge for 25

min before use (approximately $12,000 \times g$). Cells (1×10^6 to 3×10^6) were incubated with monoclonal antibodies at an appropriate dilution for 30 min at 0 to 4°C in a 0.05 M phosphate buffer (pH 7.2)-2% (wt/vol) bovine serum albumin-0.02% (wt/vol) sodium azide solution (PBA). Cells were washed in PBA three times and incubated with fluoresceinated goat anti-mouse immunoglobulin (TAGO, Burlingame, Calif.). Cells were washed in PBA three times, suspended in PBA, passed through nylon mesh to remove clumped cells, and analyzed in a flow cytometer.

Some of the monoclonal antibodies to class I antigens were gifts of B. Knowles, The Wistar Institute, Philadelphia, Pa. Antibody 11-4.1 detects H-2K^k (43), B22-249 detects H-2D^b (31), Y3 detects H-2K^k and H-2K^b (29), and 34-2-12S detects H-2D^d (44). Hybridomas producing mouse anti-mouse class I monoclonal antibodies to the other specificities tested were obtained from the American Type Culture Collection and grown as ascites fluid in pristane-primed BALB/c mice. Antibody 16-1-2 detects H-2K^k and D^k, 15-5-5 detects H-2D^k (44), and 20-8-4 detects H-2K^bD^b and K^d (45).

Analysis of viral proteins. To analyze viral proteins in the tumor cell lines, cells were metabolically labeled with radioactive amino acids, and the viral proteins were specifically immunoprecipitated and resolved on sodium dodecyl sulfate-polyacrylamide gels (10). Cells growing at the logarithmic phase were washed three times in HBSS and incubated in HBSS for 30 min at 37°C. Cells were suspended at 2.5×10^6 /ml in methionine-free RPMI 1640 containing 250 μCi of [³⁵S]methionine (Amersham) per ml and incubated for 2 h at 37°C. After incubation, the cells were washed three times in HBSS and suspended at 10^6 cells per ml in lysing buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 1% sodium dodecyl sulfate in 0.01 M Tris (pH 7.4)-0.15 M NaCl (10). After 30 min at 4°C, the lysate was stored at -70°C.

Lysates of 10^6 cells per ml were precleared with 1% (wt/vol) Formalin-fixed *Staphylococcus aureus* Cowan 1 at 4°C for 30 min in the presence of fetal calf serum. *S. aureus* was removed by centrifugation, antiserum was added, and samples were incubated overnight at 4°C. The goat anti-retroviral antisera were obtained from the Viral Oncology Program, National Cancer Institute, Bethesda, Md., and were previously tested for specificity by radioimmunoassay. Lots 79S-771 anti-Raucher gp70, 79S-142 anti-AKR p30, and 77S-095 anti-Raucher p30 were used. *S. aureus* (1% [wt/vol]) was added, samples were incubated for 30 min at 4°C, and the complexes were removed by centrifugation and washed three times in lysing buffer. The samples were suspended in eluting buffer of 2% sodium dodecyl sulfate-5% 2-β-mercaptoethanol-62.5 mM Tris hydrochloride (pH 7.4)-10% glycerol-0.001% Coomassie brilliant blue and heated to 100°C for 2 min.

Slab gel electrophoresis was performed as described previously (30) with 5% stacking gels and 11% running gels. The gels were stained with Coomassie brilliant blue in a solution of glacial acetic acid-methanol-water (1:4:5), destained in the same solution without dye, treated with En³Hance (New England Nuclear Corp., Boston, Mass.), rehydrated, and dried. Dried gels were exposed to X-ray film (Kodak XAR-5) at -70°C for various times.

IUDr induction of proviruses. Nonproducer cell lines were incubated in 200 μg of IUDr per ml (18) in the dark at 37°C for 24 h; cells were washed, resuspended in growth medium, and cocultured with DEAE-dextran-treated SC-1 cells to amplify any induced viruses. Nonadherent cells used in the cocultivation were removed after 24 h by washing three

TABLE 1. Virus production and *H*-2 class I antigen expression in tumor cell lines induced with FV

Cell line ^a	<i>H</i> -2 ^b	Name ^{b,c}	Infectivity		Tropism	RT	Class I antigen expression		
			XC assay ^d	Spleno- megaly ^e			K ^f	D	KorD
15	<i>k</i>	BALB.K-FV-1	>100	3/3	NB	+	+	ND ^g	+
22	<i>k</i>	BALB.K-F112-A	40	2/3	NB	ND	+	ND	+
26	<i>k</i>	B10.K-FV-A	>100	ND	NB	ND	ND	ND	ND
14	<i>k</i>	BALB.K-F112-B	0	0/3	NA ^g	ND	+	ND	+
32	<i>k</i>	BALB.K-F115-A	30	3/3	NB	+	ND	ND	ND
35	<i>k</i>	BALB.K-F115-B	14	3/3	NB	+	ND	ND	ND
36	<i>k</i>	BALB.K-F115-C	0	0/3	NA	-	ND	ND	ND
42	<i>k</i>	BALB.K-F129-A	>100	3/3	NB	+	ND	ND	ND
43	<i>k</i>	BALB.K-F129-B	49	3/3	NB	+	ND	ND	ND
9	<i>b</i>	Hfl/b-tc (BALB.B)	0	0/2	NA	-	+	+	ND
17	<i>b</i>	BALB.B-F50-D	0	0/3	NA	ND	+	+	ND
10	<i>b</i>	B10/Li-F50-A	0	0/3	NA	ND	+	+	ND
13	<i>b</i>	BALB.B-F113-C	0	0/3	NA	ND	+	+	ND
8	<i>b</i>	BALB.B-F113-D	>100	6/6	NB	+	+	+	+
52	<i>b</i>	BALB.B-FV129-A	0	0/3	NA	ND	ND	ND	ND
53	<i>b</i>	BALB.B-FV129-B	0	0/3	NA	ND	ND	ND	ND
4	<i>d</i>	Hfl/d-tc (BALB/c)	0	0/3	NA	-	+	+	ND
19	<i>d</i>	BALB/c-F97-A	0	0/3	NA	+	+	+	ND
12	<i>d</i>	BALB/c-F97-c	0	0/3	NA	ND	+	+	ND
21	<i>d</i>	BALB/c-F114-A	>100	4/4	NB	+	-	-	ND
18	<i>d</i>	BALB/c-F97-B	0	ND	NA	ND	ND	ND	ND
39	<i>d</i>	BALB/c-F115-A	0	0/3	NA	-	ND	ND	ND
45	<i>d</i>	BALB/c-F115-B	0	0/3	NA	-	ND	ND	ND
47	<i>d</i>	BALB/c-F115-C	0	0/3	NA	-	ND	ND	ND
48	<i>d</i>	BALB/c-F115-D	0	0/3	NA	-	ND	ND	ND
57	<i>kd</i>	(B.K × B/c)F ₁ -FV-1 ^h	0	0/3	NA	-	ND	ND	ND
58	<i>kd</i>	(B.K × B/c)F ₁ -FV-2	0	0/3	NA	-	ND	ND	ND
59	<i>kd</i>	(B.K × B/c)F ₁ -FV-3	0	0/3	NA	-	ND	ND	ND
60	<i>kd</i>	(B.K × B/c)F ₁ -FV-4	0	0/3	NA	+/-	ND	ND	ND
61	<i>kd</i>	(B.K × B/c)F ₁ -FV-5	17	2/3	NB	+	ND	ND	ND
62	<i>kd</i>	(B.K × B/c)F ₁ -FV-6	0	0/3	NA	-	ND	ND	ND
63	<i>kd</i>	(B.K × B/c)F ₁ -FV-7	0	0/3	NA	-	ND	ND	ND

^a All cell lines reported now to be nonproducers were originally found to be nonproducers within 1 year of explantation. Culture times (years) for cell lines prior to study: >1:12,13,14,18,19,21,22,32,35,36,39,42,43,47,48,52,53,57,58,59,60,61,62, and 63; >2:10 and 17; >4:15; >9:4 and 9.

^b Chi-square values for producers versus nonproducers: all *H*-2^k versus all *H*-2^b, *P* < 0.01; all *H*-2^k versus all *H*-2^d, *P* < 0.005; BALB.K versus BALB.B, *P* < 0.05; BALB.K versus BALB/c *P* < 0.01; BALB.K versus (BALB.K × BALB/c)F₁, *P* < 0.025.

^c The names read as follows: (i) the mouse strain in which the tumor was induced, except for cell lines 4 and 9 where the strains of origin are listed in parentheses; (ii) the virus used to induce the tumor either FV or F followed by a number indicating the virus preparation; (iii) a letter or number distinguishing cell lines arising from different primary tumors.

^d XC plaques per milliliter of cell culture supernatant in which 10⁶ viable cells were growing for 18 h. The nonproducer phenotypes of cell lines with XC-negative supernatants were confirmed in infectious center assays.

^e The results are listed as incidence of splenomegaly, because the positives all had greatly enlarged spleens with confluent foci.

^f Monoclonal antibodies were used to detect the various *H*-2 class I molecules: (i) for the *H*-2^k cell lines, 11-4.1 for *H*-2^k, 15-5.5 for *H*-2D^k, and 16-1-2 for either *H*-2K^k or *H*-2D^k; (ii) for the *H*-2^b cell lines, Y3 for *H*-2K^b, 34-2-12 for *H*-2D^b, and 20-8-4 for either *H*-2K^b or *H*-2D^b; (iii) for the *H*-2^d cell lines, 20-8-4 for *H*-2K^d and 34-2-12 for *H*-2D^d.

^g NA, Not applicable; ND, not determined.

^h (B.K × B/c)F₁ = (BALB.K × BALB/c)F₁.

times. The adherent SC-1 cells were passed several times and tested for the presence of virus in the XC assay. Supernatants from these cultures were passed through 0.45-μm-pore filters, and virus was analyzed for tropism on embryo fibroblasts with different *Fv-1* genotypes.

Superinfection. Cell-free FV was obtained from filtered (0.20 μm) culture supernatants of confluent BALB.K-FV-1 (cell line 15) tumor cells. These supernatants were used to infect cultured nonproducer tumor cells treated 24 h previously with Polybrene (4 μg/ml). Control cells were incubated with Polybrene and given supernatant from cell line 14 (a nonproducer cell line). These tumor cells were then passaged for 1 week, at which time culture supernatants were harvested and used in the spleen focus assay (2) with NFS

(N type) and BALB/c (B type) mice. Dilutions of the supernatant were tested to give countable numbers of spleen foci.

RESULTS

Determination of virus production. The XC assay was used to test supernatants of the various cultured tumor cell lines for the presence of shed infectious FV (Table 1). Seven out of nine cell lines derived from mice expressing the *H*-2^k haplotype (Table 1) continuously produced infectious virus in cultures (producer cells). Cell line 26 was derived from B10.K mice, and the others were derived from BALB.K mice. In contrast, one of the seven cell lines derived from mice expressing the *H*-2^b haplotype (Table 1), one of which

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of viral proteins from tumor cell lines. The lane numbers correspond to cell line numbers. Cells were metabolically labeled with [35 S]methionine and precipitated with either goat anti-p30 (A) or goat anti-gp70 (B) antiserum.

was from a C57BL/10 mouse, and one of nine cell lines derived from mice expressing the $H-2^d$ haplotype (Table 1) were producing infectious murine leukemia virus (non-producer cells). In addition, failure to produce virus was observed in six out of seven (BALB.K $\times$ BALB/c) F_1 FV-induced tumor cell lines, a failure similar to that observed in tumor cell lines derived from parent BALB/c mice; thus, the failure to produce virus continuously appears to be a dominant trait. These observed differences between $H-2^k$ tumor cell lines and $H-2^b$, $H-2^d$, and $H-2^{b/d}$ lines are statistically significant (Table 1, footnote b). The nonproducer phenotype of cell lines with XC-negative supernatants was confirmed in an infectious center assay, using 10^6 viable tumor cells to infect the fibroblasts (data not shown). In addition, inoculation of susceptible animals with supernatants from nonproducer cultures caused no disease, which typically occurred when supernatants from virus-producer cell lines were used (Table 1).

Although not all of the cell lines were tested for virus production upon initial explantation, those nonproducer cell lines which had been tested early in their passage history (cell lines 4, 9, 13, 19, 36, 39, 45, 47, 48, 52, 53, 57, 58, and 62) were producing virus upon explantation and stopped

producing virus during in vitro passage. Cessation of virus production appeared to be a relatively stable event, because no nonproducer cell lines were found to revert to the producer phenotype, although sporadic low levels of infectious virus were observed in cell line 13 and have been reported previously in cell line 4 (4).

The virus shed by the producer tumor cell lines was determined by two of its properties to be the exogenous virus used to induce the tumor in vivo. (i) The virus harvested from the supernatants induced a rapid splenic enlargement typical of Friend disease. (ii) The virus retained the NB tropism of the inducing virus, as demonstrated by the fact that the virus produced approximately the same number of XC plaques when tested on cells from $Fv-1^{b/b}$, $Fv-1^{n/n}$, and heterozygous $Fv-1^{n/b}$ mice (Table 2, cell line 15; data for the other producer cell lines are not shown). Because all endogenous viruses are either N or B tropic (58), the NB-tropic virus in the supernatants must have been the progeny of the original exogenous virus used for infection in vivo.

Alterations in viral proteins in nonproducer cell lines. Protein expression in cell lines in this study was analyzed by specific immunoprecipitation of virus proteins from metabolically labeled cells with anti-p30 and anti-gp70 antisera (Fig. 1). The producer cell lines (cell lines 8, 15, 21, and 22) appeared to make the expected viral proteins gPr95^{gag}, gPr85^{gag}, Pr65^{gag}, p30, Pr180^{gag-pol}, Pr90^{env}, and gp70 (13). As has been described previously for virus-producing primary tumor cells (15), gp70 was difficult to detect in the producer cell lines, and p30 was not always readily seen but could usually be identified by overexposing the gels. The spleen focus-forming virus-encoded gp52/55 was found in all of the FV-derived cell lines regardless of the producer status (Fig. 1) (only gp52 is observed in cell lines 9, as previously described [1], and 21).

In contrast to the producer cell lines, at least one defect in viral protein expression was observed in each of the nonproducer cell lines (Fig. 1). The most common type of defect appeared to be the failure to make precursor polyproteins. No protein corresponding to Pr65^{gag} was seen in cell line 13, no proteins corresponding to Pr90^{env} were detected in cell line 10, 12, or 14, and neither of these precursor polyproteins was seen in cell line 4, 9, or 17 (Fig. 1). Of the cell lines which did not have detectable Pr65^{gag}, however, cell lines 4 and 17 appeared to express anti-p30 precipitable proteins of 85 or 95 kilodaltons or both, (21) which are usually associated with the cell membrane (14, 60).

Some of the nonproducer cell lines expressed aberrant viral proteins. In cell lines 10, 12, and 14, the proteins which appeared to correspond to Pr65^{gag} were small compared with producer cells (Fig. 1). Additionally, cell line 18 expressed a protein of approximately 36 kilodaltons which was precipitated by anti-p30 antiserum and two low-molecular-weight proteins precipitated by anti-gp70 antiserum (data not shown).

To determine if the protein profiles of these nonproducer cell lines reflected either identical defects which occurred in each clone of the line or a composite of defects expressed in different clonal populations, the production of viral proteins was examined in eight cloned tumor cell lines derived from cell lines 13 ($H-2^b$) and 12 ($H-2^d$) (four clones from each parent cell line). The protein profile of each of these clones was identical to that observed in the parent cell line (data not shown), suggesting that each clone in these nonproducer tumor cell lines expressed the same defect in viral protein production.

The changes in viral protein expression observed in the nonproducer cell lines were highly variable, and no single change was observed which was associated with all of the nonproducer cell lines nor were any haplotype-specific changes identified. The extent and variety of the alterations found in viral proteins in these cells and others (1, 36, 49, 63), including clonal variations (4, 9), suggest that their expression may be highly unstable. In contrast, precipitations performed on the same cell line at various intervals extending over a period of several years after the cells have become nonproducers have demonstrated that after the initial aberrations were first observed, no further changes occurred (data not shown). This apparent stability may reflect the emergence of dominant nonproducer clones which are under no selective pressure for change once the *gag* and the *env* pathways have been altered.

H-2 class I antigen expression. It was of interest to determine if any relationship existed in these cell lines between the expression or production or both of virus and class I H-2 encoded molecules. Some class I molecules become incorporated into FV particles, indicating that these molecules may be physically associated with viral proteins (8). We did not observe any apparent class I major histocompatibility complex proteins coprecipitating with viral antigens (Fig. 1), nor have any apparent viral antigens been coprecipitated with class I molecules from these cell lines (B. Spear and K. Blank, unpublished observations). Thus in these studies there was no evidence for a strong interaction between these proteins.

Expression of class I molecules in these FV-induced erythroleukemia cell lines appeared to be normal (with the exception of cell line 21), in contrast to previous studies in which altered cell surface expression of class I major histocompatibility complex antigens has been reported in several cultured lymphoid tumor cell lines in vitro (28, 39, 48, 52, 53).

Inducibility of the exogenous provirus in nonproducer cell lines. Altered provirus DNA might have produced the defects in virus production and virus protein expression observed in these FV-induced tumor cell lines (12, 55). To determine if the provirus of the exogenous virus was still present in the genome of these tumor cells in a form which could lead to the production of a biologically active virus, several nonproducer tumor cell lines were treated with IUdR and tested for the production of exogenous, NB-tropic virus. Four out of the five nonproducer tumor lines tested began producing NB-tropic virus (Table 2), suggesting that the proviruses of the original exogenous NB-tropic viruses used for infection in vivo were not altered in these nonproducer cell lines in a manner which inhibited induction by IUdR.

TABLE 2. Replication in fibroblasts of viruses recovered after IUdR treatment of nonproducer cell lines

Cell line	XC plaques/ml of culture supernatant in fibroblasts with <i>Fv-1</i> genotype:		
	BALB.K-ef <i>b/b</i>	(BALB/c × NFS) <i>F₁</i> -ef <i>b/n</i>	NIH-3T3 <i>n/n</i>
15 ^a	64	84	92
4	144	103	166
13	325	28	3
17	103	106	114
18	225	163	107
19	280	141	165

^a Supernatants from untreated producer cell line 15 were tested in the same assay and are included for comparison.

TABLE 3. Superinfection of nonproducer FV-induced cell lines

Cell line	Haplotype	Treatment	Spleen foci ^a in:	
			BALB/c	NFS
14	<i>k</i>	None	0 ± 0	0.7 ± 1.2
	<i>k</i>	FV	31.7 ± 9.0	21.7 ± 7.2
36	<i>k</i>	None	0.7 ± 0.6	0 ± 0
	<i>k</i>	FV	38.3 ± 6.7	11.3 ± 3.1
17	<i>b</i>	None	0 ± 0	1.0 ± 1.0
	<i>b</i>	FV	8.0 ± 7.9	33.3 ± 8.0
13	<i>b</i>	None	1.0 ± 1.0	0 ± 0
	<i>b</i>	FV	16.3 ± 3.8	34.0 ± 16.6
4	<i>d</i>	None	0.7 ± 1.2	1.7 ± 0.6
	<i>d</i>	FV	10.0 ± 9.8	7.7 ± 5.7
19	<i>d</i>	None	1.0 ± 0.0	0.7 ± 1.2
	<i>d</i>	FV	23.7 ± 18.1	5.0 ± 2.0

^a Groups of three mice were inoculated with supernatant. Foci reported are the mean ± standard deviation.

Superinfection of nonproducer cell lines. Previous studies have suggested that producer tumor cell lines may become nonproducer lines during in vitro passage as the result of the spontaneous emergence of nonproducer clones which, as the consequence of some unknown selective advantage, overgrow the producer cells. If this were the case in this system, the failure of tumor cell lines from *H-2^k* mice to stop producing virus might indicate that upon the emergence of nonproducer clones these cells are superinfected by virus from neighboring producer cells resulting in the reinitiation of virus production. It thus seemed possible that the reason for the cessation of virus production in cell lines derived from *H-2^b* and *H-2^d* mice was that these cells, by virtue of the expression of some *H-2*-encoded molecule on the cell surface, were resistant to superinfection.

To test this possibility, nonproducer FV-induced tumor cells were incubated with cell-free supernatants from an *H-2^k* producer tumor cell line and examined for reinitiation of virus production in the spleen focus assay. The results indicated that *H-2^d*, *H-2^b*, and *H-2^k* nonproducer cell lines upon superinfection began once again to shed infectious virus (Table 3). Therefore, the cell surface *H-2* molecules expressed by particular tumor cells did not seem to affect the ability of these cells to become superinfected.

DISCUSSION

The data presented in this report suggest that a host cell gene(s) linked to *H-2* controls production of exogenous retroviruses in cultured tumor cell lines induced by FV; i.e., tumor cells expressing the *H-2^k* haplotype continue to produce infectious progeny virus, whereas those expressing the *H-2^b* or *H-2^d* haplotype ceases to produce virus. The cessation of virus production was observed to be a dominant trait in tumor cells derived from *H-2^{k/d}F₁* mice. In the few cell lines with non-BALB/c backgrounds, virus expression showed the same relationship to *H-2* haplotype expression as in the BALB-derived cell lines. This control does not appear to be exerted at the DNA level through alteration of the provirus, since FV production could be induced in nonproducer cells by treatment with IUdR. The facts that all tumor cells examined upon explantation regardless of *H-2* haplotype expression were initially producing virus and that cessation of virus production occurred during in vitro pas-

sage suggest that control of virus production is an intracellular event unrelated to the immune response of the host to virus determinants. Thus it appears possible that this control may be exerted at the level of transcription, translation, or posttranslational modification. In fact, evidence for cessation of transcription of the Friend murine leukemia virus component of FV has been described previously in cell lines 4 and 9 (3). The continued expression of viral proteins, however, especially gp55/52 which is spleen focus-forming FV specific, indicates that transcription of some viral proteins continues in all the cell lines regardless of their *H-2* haplotype.

The apparent randomness in the phenotypic expression of viral proteins in these tumor cell lines and among clones from nonproducer cell lines (4, 10) suggests that the observed influence on virus expression may be secondary to some intracellular reaction mediated by the *H-2*-linked gene(s). This event may be some normal cellular biochemical event which is utilized by the virus for its expression. One candidate for this role might be a gene encoding a product involved in some step in a glycosylation pathway because both viral and *H-2* proteins are glycosylated, and genes encoding enzymes associated with glycosylation have been mapped within or near *H-2* (16, 56).

Because the *H-2^k* haplotype is associated with both susceptibility to FV in vivo and virus production in vitro, this apparent *H-2*-mediated direct effect on virus replication may be important in leukemogenesis (62). A necessary hypothesis then is that this control of virus production in cultured tumor cell lines induced with high doses of virus reflects the events which occur in vivo with low virus doses at which differences in *H-2*-linked resistance are observed (33, 46). Accordingly, when mice are inoculated with low doses of murine leukemia virus, target cells in *H-2^k* mice may begin to produce virus which causes recruitment of new tumor cells and severely affects the anti-tumor immune response as described below, whereas target cells in resistant *H-2^b* mice may repress virus production enabling them to generate an effective immune response to virus-infected cells. It is unlikely that this *H-2*-linked genetic control of virus production is the only *H-2*-linked effect which governs susceptibility. If it were the only such control, those haplotypes associated with cessation of virus production would also be associated with resistance to that virus. However, the *H-2^d* haplotype, which has been demonstrated in these studies to be associated with the cessation of virus production in FV-induced tumor cells, is associated with susceptibility to FV. Thus in *H-2^d* mice, virus production may be inhibited when small doses of virus are given for infection, but there may be a failure in the ability of the animal to generate an effective immune response to those cells which have become infected (and ultimately transformed) by FV because of the expression of an *H-2*-linked immune response gene. This same immune response gene would conceivably be responsible for an effective anti-FV response in *H-2^b* mice. It is also important to note that not all *H-2^k* cell lines are producers, and not all the *H-2^b* and *H-2^d* lines are nonproducers, which may reflect the original observations in vivo that *H-2*-mediated resistance was only relative, not absolute.

Several previous observations related to *H-2* and the expression of retroviruses or retrovirus-related proteins suggest that *H-2*-linked genes may exert a regulatory influence on provirus expression in untransformed cells. (i) The *H-2*-linked *Cvx-1* gene appears to control the level of expression in vivo of xenotropic virus from the *Bvix-1* locus on chromosome 1, and no immune responses were found which could

have accounted for this control (64). (ii) In somatic cell hybrids, chromosome 17 (which encodes the *H-2* complex) appears to be necessary but not sufficient to permit replication of exogenous ecotropic (20, 51) and mink cell focus-forming (20) viruses. (iii) Expression of the *G_{IX}* antigen, associated with endogenous gp70 molecules, segregates with both *H-2* on chromosome 17 and *Fv-1* on chromosome 4 (57). (iv) *H-2* genes appear to influence the transcription of endogenous spleen focus-forming FV-related sequences in spleen cells from uninfected mice (35).

There are several ways in which altered expression of the exogenous retrovirus by the host cell could potentially affect processes which are thought to be involved in leukemogenesis. (i) Immune responses have been shown to be affected by the presence of virus particles (6, 47) and by alteration of viral proteins on the cell surface (25). Viral protein alterations have been observed in all nonproducer tumor cell lines examined (Fig. 1) (1, 10, 36, 47, 49, 63), which suggests that infected host cells may be capable of altering their antigenic expression. (ii) The binding of recombinant viruses to cell surface receptors may contribute to the leukemogenic process through chronic mitogenic stimulation of target cells (37). Altering the level of virus production could change the amount of mitogenic stimulation thus altering the final incidence of leukemia. (iii) The *H-2*-mediated control of virus production may affect the spread of the virus infection in the host. This effect would result in a reduction in the number of target cells transformed as the result of infection. Because *H-2*-mediated control of leukemogenesis is only observed with relatively low virus doses (33, 46), it would appear that inoculation of high virus doses may overcome these possible effects.

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