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Mechanism of Induction of Class I Major Histocompatibility Antigen Expression by Murine Leukemia Virus

Douglas V. Faller, Lise D. Wilson, and David C. Flyer

Division of Pediatric Oncology, Dana Farber Cancer Institute and Department of Pediatrics, Harvard Medical School and Childrens Hospital Medical Center, Boston, Massachusetts 02115 (D.V.F., L.D.W.), Department of Microbiology, Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033 (D.C.F.)

Alterations in expression of major histocompatibility complex (MHC) antigens on tumor cells clearly correlate with the tumorgenicity and metastatic potential of those cells. These changes in the biological behavior of the tumor cells are presumably secondary to resulting changes in their susceptibility to immune recognition and destruction. Murine leukemia viruses (MuLV) exert regulatory effects on class I genes of the MHC locus. MuLV infection results in substantial increases in cell surface expression of all three class I MHC antigens. These viral effects on MHC antigen expression profoundly influence immune-mediated interaction with the infected cells, as assessed by cytotoxic T lymphocyte recognition and killing. Control of class I MHC and beta-2 microglobulin genes by MuLV takes place via a *trans*-acting molecular mechanism. MuLV controls expression of widely separated endogenous cellular MHC genes, transfected xenogeneic class I MHC genes, and unintegrated chimeric genes consisting of fragments of class I MHC genes linked to a bacterial reporter gene. These findings indicate that MuLV exerts its effects on MHC expression via a *trans* mechanism. The MuLV-responsive sequences on the MHC genes appear to lie within 1.2 kilobases upstream of the initiation codon for those genes.

Key words: immune surveillance, *trans* activation, retroviruses, class I MHC antigens, leukemia viruses

The level of cellular immune response against tumors appears to be a major factor in determining tumor growth and metastatic behavior [1–6]. Any process which alters immune recognition may therefore alter the response of the organism to the tumor, either facilitating the interaction of the tumor with the immune system or permitting the escape of these transformed cells from immune destruction. The class I major histocompatibility complex (MHC) antigens are polymorphic cell surface glycoproteins that function as targets, directing the attack of cytotoxic T lymphocytes

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(CTL) against virally transformed cells. CTL function and recognition of target cells require associated recognition between foreign antigens and self-MHC components [6–8]. In the case of lymphomas induced by murine leukemia viruses, these foreign antigens can be either virally encoded proteins or nonviral tumor-specific antigens induced in the course of tumorgenesis [9,10]. The level of expression of MHC class I proteins on virus-infected cells has been correlated with the degree of effectiveness with which they are recognized by CTL [11,12]. On some tumors, the new or enhanced expression of specific class I MHC antigens is associated with increased potential for invasion and metastasis [13]. It is, therefore, reasonable to expect that viruses with the capability of altering class I MHC expression in the cells they infect may be influencing their own survival and the survival of the cells they transform.

Previously, this laboratory has reported that mouse cells express significantly increased surface levels of murine class I MHC H-2K, H-2D, and H-2L proteins after infection with the Moloney murine leukemia virus (M-MuLV) [11]. The infected cells are efficiently lysed by M-MuLV-specific CTL and also by allospecific CTL, a reflection of their increased level of H-2 antigens. Coinfection of cells with M-MuLV and the replication-defective, acutely transforming Moloney murine sarcoma virus (M-MSV) eliminates the H-2 enhancement as well as decreasing the immunosensitivity of the infected cells.

This report describes our efforts to define the mechanism by which the murine leukemia viruses induce class I MHC expression. Our findings indicate that as a result of MuLV infection, cells increase their synthesis of MHC class I mRNA, as well as beta-2 microglobulin mRNA. Analysis of MuLV-infected cells transfected with MHC class I genes suggests that the viral regulation of H-2 transcription occurs in *trans*. We also show that sequences upstream of at least one murine class I gene are responsive to this *trans* regulation and will direct increased production of an attached bacterial reporter gene in M-MuLV-infected cells.

MATERIALS AND METHODS

Viruses

Moloney murine leukemia virus clone 1 [14] was obtained from Drs. C. Tabin and R. Weinberg. In some experiments the source of the virus was a producer cell line of BALB/c-3T3 cells transfected with an infectious proviral clone of Moloney MuLV designated pMoV-9 [15], or with an infectious proviral clone of an amphotropic MuLV, strain 4070, kindly provided by Dr. A. Oliff.

Cells

BALB/c-3T3 fibroblasts and NIH-3T3 fibroblasts were obtained through the American Type Culture Collection (Rockville, MD). LB10SV, a fibroblast cell line derived from C57BL/10 mice, was described previously [9]. Infected and uninfected fibroblasts were cultured in Dulbecco's modified Eagle's medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS) and 2 mM L-glutamine.

Monoclonal Antibodies

Hybridoma cell lines producing monoclonal antibodies specific for H-2K^d (31.3.45), H-2D^d (34.2.12), H-2L^d (28.14.8), and H-2K^b (B8-24-3) were provided

by Dr. David Sachs. Monoclonal antibody specific for HLA-A2 was obtained from hybridoma PA2.5 (American Type Culture Collection, Rockville, MD). Cell-free supernatants from hybridoma-conditioned medium were used undiluted for cell surface antigen labeling. An antisera raised in rabbits against purified H-2^k, which cross-reacts with all murine H-2 proteins, was the generous gift of Dr. S. Herrmann, and was used as a 1:25 dilution for cell staining.

Concanavalin A (Con A) Supernatant

Spleen cells from Lewis rats (Charles River Laboratories, Wilmington, MA) were cultured in the presence of 5 μg/ml Con A (Pharmacia Fine Chemicals, Uppsala, Sweden) for 24 h. The cell-free supernatant was partially purified by ammonium sulfate precipitation as described [11,16].

Generation of CTL

Allospecific CTL (anti-H-2^b) were generated in 5-day, one-way mixed lymphocyte cultures. BALB/c spleen cells (5×10^6) were cultured with an equal number of gamma-irradiated (2,500 rads) C57BL/6 spleen cells in 16-mm Linbro plates (Flow Laboratories, Inc., McClean, VA), in 2 ml of RPMI 1640 (GIBCO), supplemented with 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, and 10% FCS. Anti-H-2^d allospecific CTL were generated in an analogous way, except that irradiated BALB/c spleen cells were used as stimulators and C57BL/6 spleen cells were used as the responding population.

^{51}Cr-Release Assay

^{51}Cr-release assays were carried out in duplicate in 96-well V-bottomed microtiter plates. Varying numbers of immune lymphocytes were added to 10^4 ^{51}Cr-labeled target cells in 0.2 ml wells and were incubated for 4 h. After incubation, the cells were pelleted, and 50% of the well supernatant was removed and was counted in a gamma counter (Packard Instrument Co., Grove, IL). Percent specific ^{51}Cr release was calculated as follows:

$$\frac{(^{51}\text{Cr release with immune lymphocytes}) - (\text{spontaneous } ^{51}\text{Cr release})}{(\text{Maximum } ^{51}\text{Cr released with 3\% Triton}) - (\text{spontaneous } ^{51}\text{Cr release})} \times 100$$

Immunofluorescent Labeling and Analysis

Live cells were incubated with hybridoma culture supernatants followed by Fluorescein-conjugated, affinity-purified F(ab')$_2$ fragment of sheep antimouse IgG (Cooper Biomedical, Malvern, PA), as described [11]. After fixation in 2% paraformaldehyde, labeled cells were analyzed by an Epics V fluorescence-activated cell sorter (Coulter Electronics, Inc., Hialeah, FL). As a control, some cell populations were exposed to recombinant murine interferon gamma (Genentech, South San Francisco, CA), at a concentration of 100 U/ml for 48 h prior to staining.

Northern Blot Analysis of Total Cellular RNA

Total cellular RNA was extracted from cells using a modification of the guanidine hydrochloride extraction technique described by Strohman et al. [17]. Briefly,

after three serial ethanol precipitations in the presence of 6 M guanidine/100 mM potassium acetate, the nucleic acids were extracted with a phenol/chloroform mixture and ethanol precipitated. Twenty micrograms of RNA were loaded per lane on a 1.2% agarose gel containing formaldehyde and ethidium bromide [18]. Following electrophoresis, transfer to nitrocellulose was carried out using standard techniques. After baking in vacuo, prehybridization of the filters was performed in 5 × SSC (0.75 M NaCl, 75 mM trisodium citrate, pH 7.0) and 50% formamide at 42°C. Hybridization was carried out under the same conditions, in the presence of 10% dextran sulphate and 2×10^6 cpm of radiolabeled probe/ml of hybridization fluid. The blots were washed under stringent conditions (68°C, 0.2 × SSC) and autoradiography was carried out using intensifying screens at −70°C. Densitometric scanning (Helena Laboratories, Beaumont, TX) was performed to quantitate relative amounts of hybridization.

The probe used to detect H-2 class I sequences was a 2.5-kilobase (kb) Bam HI fragment from a plasmid clone containing the H-$2D^b$ genomic clone (kindly provided by Drs. H. Allen and R. Flavell). This fragment contains the strongly conserved exons 4–8 and the 3′ untranslated sequences and hybridizes to all murine class I mRNA species. The probe for beta-2 microglobulin was previously described [11]. Probes were labeled to a specific activity of $>10^8$cpm/μg, using the random oligo primer method [19]. In order to normalize for the amount of RNA in each lane, blots were stripped of probe following autoradiography and rehybridized with an actin cDNA probe [20].

DNA-Mediated Gene Transfer

The Human Leukocyte Antigen-A2 (HLA-A2) gene, subcloned into the Eco RI site of pBR328 [21], was kindly provided by Drs. A. Biro and J. Strominger. This DNA was coprecipitated with the selectable marker pMSV-*neo* (10, and see below) or pSV2-*neo* [22] and salmon sperm DNA as carrier. Transfection and isolation of clones was carried out as previously described [10]. Clones were selected for cell surface expression of HLA-A2 by immunofluorescent staining and analyzed by fluorescence-activated cell sorting.

CAT Vector Constructions

The subclone of the genomic H-$2K^b$ gene in pBR327 [23] was digested with the restricted enzymes Hind III and Nru I to generate a 2.1-kb fragment. After isolation, the fragment was blunt-ended with the large fragment of *Escherichia coli* DNA polymerase I and ligated to Hind III oligonucleotide linkers. The linkered fragment was then ligated into the Hind III site of the chlorampenicol acetyl transferase (CAT) vector, pSVO-CAT [24]. Transformation of competent *E. coli* HB101 resulted in Amp^R colonies which were screened by the minilysate technique [25] for insertion and correct orientation of the H-$2K^b$ promoter sequences relative to the bacterial CAT gene (pKbHN-CAT, see Fig. 4). The construction pKbPN-CAT was generated by isolating the 1.2-kb Pvu II-Nru I fragment of the H-$2K^b$ gene, ligating it to Hind III linkers and cloning it into pSVO-CAT as above (see Fig. 4). All enzymes and linkers were obtained from New England Biolabs (Beverly, MA) and were used according to the supplier's recommendations.

Assay for Transient CAT Expression

All CAT plasmids were isolated by lysozyme-SDS lysis and cesium chloride-ethidium bromide equilibrium gradient centrifugation. Cells were plated at 10^6/100-

mm tissue culture dish approximately 18 hr before transfection. Calcium phosphate precipitates containing 10 μg of the CAT vector and 20 μg salmon sperm carrier DNA were prepared, and transfections were carried out as described previously [10]. The CAT vector pSV2-CAT [24], provided by Drs. R. Mulligan and J. Nye, was used as a positive control for CAT expression in each of the cell lines tested. Forty-eight hours after transfection, the cell monolayers were washed with and scraped into ice-cold phosphate-buffered saline. The pelleted cells were resuspended in 100 μl of 0.25 M Tris-HCl, pH 7.8, and cell extracts were prepared by freezing and thawing the resuspended cells three times. Cellular debris was removed by centrifugation in a microfuge for 5 min at 4°C. The extracts were then measured for protein content and assayed for CAT activity as described by Gorman et al. [24]. Controls including purified CAT enzyme (.001 unit/assay; Pharmacia, Piscataway, NJ), instead of cell extract, were also run as standards for acetylation states of the chloramphenicol.

RESULTS

Infection of murine fibroblasts with M-MuLV results in enhancement of cell surface expression of MHC antigens. By using monoclonal antibodies specific for each of the three murine class I MHC proteins on BALB/c cells (H-2K^d, H-2D^d, and H-2L^d) it was demonstrated that the levels of all three antigens are upregulated on the surface of chronically infected BALB/c-3T3 cells (Fig. 1). The magnitude of the increase is up to tenfold for H-2K and somewhat less for H-2D and H-2L. The temporal course of this increase in class I antigen expression parallels the relatively slow spread of viral infection through newly infected cells (as monitored by expression of viral glycoprotein and positive immunofluorescence using a fluorescein isothiocyanate (FITC)-conjugated anti-viral glycoprotein monoclonal antibody). However, some increase in class I MHC expression can be demonstrated as soon as

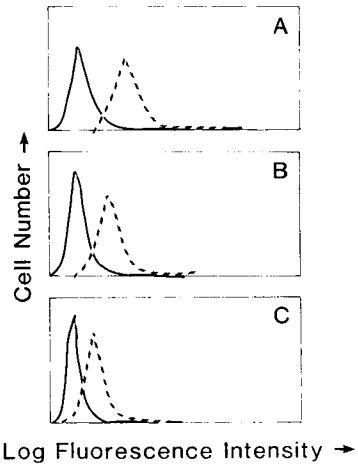

Fig. 1. Effect of murine leukemia virus infection of BALB/c-3T3 fibroblasts on cell surface class I MHC antigen expression. Binding of monoclonal antibodies specific for H-2K^d(**A**), H-2D^d(**B**), and H-2L^d(**C**) was measured by flow cytofluorometry after reacting infected or uninfected BALB/c cells with anti-H-2 monoclonal antibodies, followed by FITC-conjugated goat anti-mouse IgG. Uninfected BALB/c-3T3,(———); M-MuLV-infected BALB/c3T3, (- - - - -).

48 hr after exposure of the cells to virus-containing media (data not shown). Similarly, MuLV-infection of NIH-3T3 cells causes an increase in cell surface expression of class I MHC antigen (Fig. 2A). Because NIH-3T3 cells are not from a defined inbred and MHC-phenotyped mouse strain, a pan-reactive antimurine class I rabbit serum was initially used to detect all class I-related molecules on the surface of these cells. Figure 2B demonstrates enhanced reactivity of NIH-3T3 cells with this serum following treatment with interferon gamma. Subsequently, we found that a monoclonal antibody specific for H-2D^b would detect class I molecules induced on NIH-3T3 cells by MuLV infection (data not shown).

Another way of assessing levels of immunologically active MHC antigens on the surface of cells is by analysis of their reactivity with allospecific T lymphocytes. The susceptibility of MuLV-infected BALB/c cells to lysis by anti-H-2^d-specific CTL paralleled their increase in H-2^d expression as determined by antibody staining, in that MuLV infection resulted in a greater than fourfold enhancement in lysis by allospecific CTL (Table I). Relative susceptibility to allospecific lysis is determined by the need for a fourfold increase in the effector-to-target-cell ratio for equivalent killing of the uninfected versus the MuLV-infected BALB/c cells. Infection of BALB/c cells with another murine retrovirus, amphotropic MuLV, also resulted in increased lysis by alloreactive CTL. Similarly, MuLV-infected NIH-3T3 cells demonstrated an

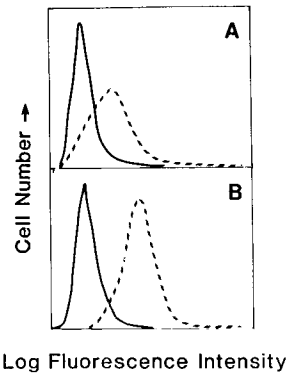

Fig. 2. Effect of murine leukemia virus infection of NIH-3T3 cells on cell surface class I MHC antigen expression. Binding of a rabbit antiserum which is pan-reactive with all murine H-2 antigens was measured by flow cytofluorometry after reacting uninfected, M-MuLV-infected, or interferon gamma-treated cells with the serum at a 1:25 dilution, followed by FITC-conjugated sheep anti-rabbit IgG. Uninfected NIH-3T3, (———). M-MuLV-infected NIH-3T3 (**A**) or interferon gamma-treated NIH-3T3 (**B**) (- - - - - -).

TABLE I. Lysis of MuLV-Infected BALB/c-3T3 Cells by Allogeneic CTL

		% Cytotoxicity[b]		
Cell line	Virus[a]	12:1[c]	25:1	50:1
BALB/c	—	33.0	43.1	57.1
BALB/c	M-MuLV	54.9	69.2	76.5
BALB/c	A-MuLV	46.3	59.3	78.3

[a]Cells were uninfected or infected with Moloney MuLV (M-MuLV) or with amphotropic MuLV (A-MuLV).
[b]Percent specific ^{51}Cr release.
[c]Effector-to-target cell ratios.

increase in their susceptibility to killing by anti-H-2^b-specific alloreactive CTL (Table II). In fact, they were lysed more efficiently than the usual H-2^b target cell line LB10SV. This finding, combined with the monoclonal antibody reactivity described above, suggests that the MHC phenotype of the NIH-3T3 cell line may contain some H-2^b-reactive elements.

To determine the molecular level at which induction of MHC by MuLV was occurring, analysis of class I-specific mRNA species in uninfected and infected BALB/c cells was carried out. The molecular probe used to detect MHC transcripts was selected on the basis of its ability to hybridize with all three types of murine class I transcripts (D, K, and L) and to cross-react to a molecular region highly conserved in all class I genes. BALB/c cells infected with M-MuLV express 4–6-fold more class I-specific transcripts than do uninfected cells (Fig. 3A). Normalization for the level of actin transcripts in infected versus uninfected cells (Fig. 3C) did not affect this ratio. Beta-2 microglobulin is a nonpolymorphic protein which associates with class I MHC polypeptides and is (in most cases) necessary for cell surface expression of MHC antigens [26]. The gene encoding beta-2 microglobulin does not lie within the MHC locus, yet the expression of beta-2 transcripts often parallels changes in the

TABLE II. Lysis of Moloney MuLV-Infected NIH-3T3 Cells by Allogeneic CTL

	% Cytotoxicity[a]			
Cell line	6:1[b]	12:1	25:1	50:1
LB10SV	13.4	29.6	38.1	52.3
NIH-3T3	7.7	8.8	14.0	18.0
M-MuLV-infected NIH-3T3	18.6	35.6	51.8	72.6

[a]Percent specific ^{51}Cr release.
[b]Effector-to-target cell ratios.

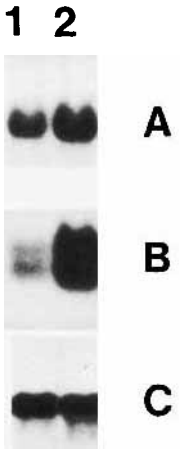

Fig. 3. Levels of class I MHC, beta-2 microglobulin, or beta-actin transcripts in BALB/c-3T3 cells, in the presence or absence of M-MuLV. Equal amounts of total cellular RNA extracted from BALB/c-3T3 cells (**lane 1**) or from M-MuLV-infected BALB/c-3T3 cells (**lane 2**) were separated on a 1.2% agarose gel, transferred to nitrocellulose, and hybridized sequentially to radiolabeled probes specific for H-2 (**A**), beta-2 microglobulin (**B**), and beta-actin (**C**) transcripts. Results of 15-hr autoradiographic exposure are shown here.

expression of class I transcripts [27]. Multiple analyses of mRNA species in infected or uninfected fibroblasts using a probe specific for murine beta-2 microglobulin revealed an average fivefold increase in the steady-state levels of both the 0.7- and 0.9-kb beta-2 microglobulin transcripts (Fig. 3B).

The finding that levels of all three class I MHC molecules and the genetically unlinked beta-2 microglobulin were elevated in M-MuLV-infected cells, and that this was reflected in an increase in the respective mRNA levels, suggested that MuLV was acting in a *trans* fashion to induce MHC and MHC-related genes. Such a *trans* mode of action should be capable of enhancement of other MHC genes which have been artificially introduced into the cell and stably integrated at loci distinct from the MHC region. To test this hypothesis, NIH-3T3 cells were transfected with the xenogeneic (human) class I MHC gene encoding HLA-A2. The resulting G418-resistant cell lines were sorted by fluoresence-activated flow cytometry and a population expressing HLA-A2 antigen on the cell surface was selected. Treatment of these transfected cells with interferon gamma resulted in an increased level of HLA-A2 expression (Fig. 4B). This heterogeneous population was then infected with M-MuLV and the cell surface levels of HLA-A2 compared to that of uninfected cells. Figure 4A demonstrates enhancement of HLA-A2 expression in the infected population.

Similarly, *trans* activation of MHC genes by MuLV should take place even in the absence of genomic integration, i.e., during transient expression of newly transfected genes. To amplify the fairly weak signal obtained during such transient gene expression assays, the 5′ upstream region of the H-2K^b gene was attached to the prokaryotic reporter gene CAT, which encodes the bacterial enzyme chloramphenicol acetyl transferase. The initial chimeric gene construction (called pKbHN-CAT, Fig. 5) included 2.1 kb of upstream sequence, terminated at an Nru I site 19 base pairs (bp) upstream of the ATG codon of the exon encoding the H-2K^b leader peptide, and thus contained the essential sequences for effective promoter activity (i.e., TATA box, CCAAT box, and CAP site). Upon transfection of this recombinant gene into

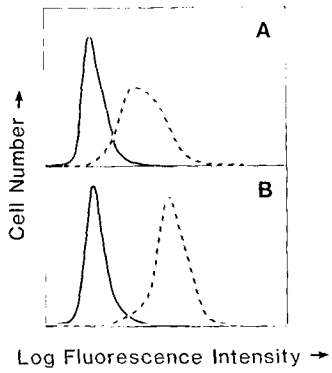

Fig. 4. Effect of murine leukemia virus infection of NIH-3T3 cells transfected with the HLA-A2 gene on the expression of HLA-A2. NIH-3T3 cells were transfected with the HLA-A2 gene and cell lines which stably expressed the A2 antigen were selected. Binding of a monoclonal antibody specific for HLA-A2 was measured by flow cytofluorometry after reacting uninfected, M-MuLV-infected, or interferon gamma-treated A2-transfected cells with the antibody, followed by FITC-conjugated goat anti-mouse IgG. Uninfected A2-transfected cells, (———). A2-transfected cells after infection with M-MuLV (**A**); or after treatment with interferon gamma (**B**), (– – – –).

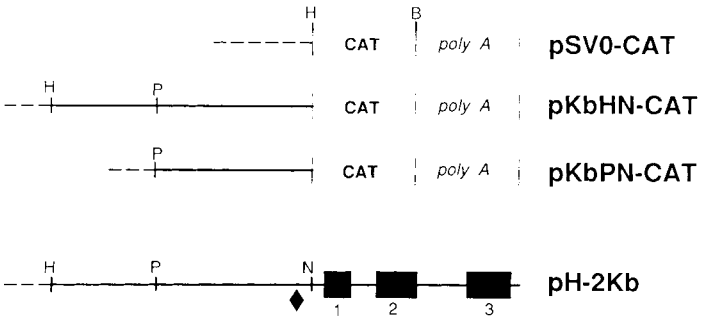

Fig. 5. Construction of pKbHN-CAT and pKbPN-CAT. The restriction map of the 5′ portion of the H-2K^b gene showing the location of the promoter (♦) and exons 1-3 (■) has been adapted from Allen et al. [23]. H = Hind III; B = Bam HI; P = Pvu II; N = Nru I. The Nru I site is located 19 bp upstream from the ATG of the H-2K^b exon 1 (leader sequence). The 2.1 kb Hind III-Nru I fragment, and the 1.2 kb Pvu II-Nru I fragment, both from the H-2K^b 5′ region (heavy lines), were ligated to Hind III oligonucleotide linkers and cloned into the Hind III site of the pSVO-CAT vector. Plasmid sequences are indicated by the broken lines. The *E. coli* Tn 9 chloramphenicol acetyl transferase gene (CAT) and the simian virus 40 (SV40) fragment containing the poly A addition site (poly A) are indicated.

uninfected, or M-MuLV-infected, BALB/c-3T3 cells and assay of cytoplasmic CAT activity at 48 hr, we found a consistent 4–5-fold increase in enzyme activity in the cells containing the leukemia virus (Fig. 6A). Normalization of this data to results obtained when the control plasmid, pSV2-CAT, was introduced into the same cell lines did not alter this ratio and negligible CAT activity was detected when the promoterless plasmid pSVO-CAT was introduced into either cell line. The relative increase in CAT expression in M-MuLV-infected cells compared to uninfected cells did not vary with time of incubation or with the amount of extract used in the incubation (data not shown).

The finding that a chimeric gene containing just the upstream elements of a class I MHC gene was controlled by M-MuLV in a fashion similar in direction and magnitude to the viral control of endogenous class I MHC genes allowed us to begin mapping those K^b control sequences which are responsive to murine leukemia virus. A second vector containing only 1.2 kb of K^b upstream sequence, and designated pKbPN-CAT (Fig. 5), was constructed and tested for activity in the same cell lines as the pKbHN-CAT vector. The difference in CAT expression driven by pKbPN-CAT in M-MuLV-infected versus uninfected cells was on average 2.5-fold (Fig. 6B). These results suggested that the MuLV effect on class I MHC expression may be mediated through an interaction somewhere within the 2.1 kb of DNA upstream from the coding sequence for the class I proteins. Shortening that sequence to 1.2 kb decreases the magnitude of the observed effect but does not eliminate it.

DISCUSSION

Abnormal regulation of MHC genes has been observed in tumors generated in a number of diverse ways, including chemically transformed cells, radiation-induced tumor cells, and spontaneously occurring leukemia cells in high-incidence mouse strains [reviewed in 28]. Attempts have been made to correlate changes in MHC expression with the metastatic or tumorgenic potential of the transformed cell [reviewed in 29]. We have shown here, and in previous studies [11], that cells in culture

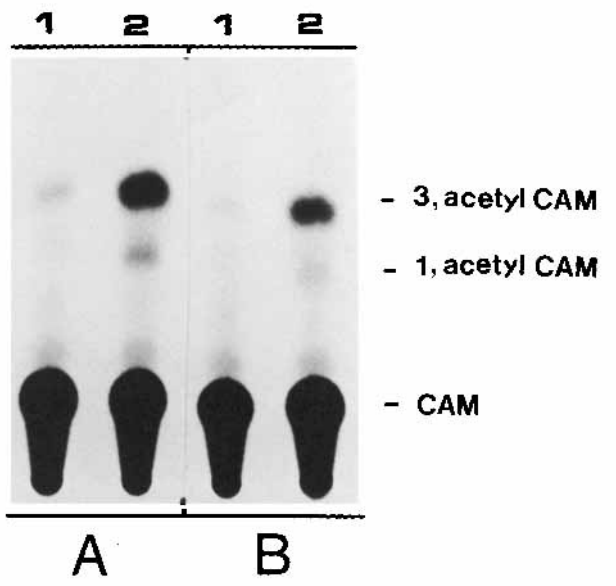

Fig. 6. Effect of leukemia virus infection on the level of CAT expression directed by pKbHN-CAT or pKbPN-CAT. Uninfected (**lane 1**), or M-MuLV-infected (**lane 2**), BALB/c-3T3 cells were transfected with calcium phosphate precipitates including 10 μg of pKbHN-CAT (**A**) or pKbPN-CAT (**B**). Cell extracts were prepared 48 hr post-transfection and assayed by incubating 500 μg of extract with 1 μCi ^{14}C-chloramphenicol for 2 hr at 37°C. The reaction products were separated by thin-layer chromatography (TLC). The autoradiograph of the TLC plate is shown with the positions of the unacetylated chloramphenicol (CAM) and acetylated reaction products (1-acetyl CAM and 3-acetyl CAM) indicated. Spots were cut from the plate and counted by liquid scintillation counting to determine the percent conversion of chloramphenicol to its acetylated derivatives. Percent chloramphenicol conversion for pKbHN-CAT-transfected, uninfected cell extracts was 0.15 $\pm$ 0.02, and for M-MuLV-infected cell extracts was 0.68 $\pm$ 0.05. Percent conversion for pKbPN-CAT-transfected, uninfected cell extracts was 0.16 $\pm$ 0.03, and for M-MuLV-infected cell extracts it was 0.37 $\pm$ 0.04.

increase their surface expression of class I MHC proteins shortly after infection with MuLV. It is highly unlikely that the increase is due to de novo expression of previously unexpressed class I genes, or to recombinational events leading to the formation of novel class I-like proteins, because increased levels of all three of the BALB/c class I antigens (H-2K, -L, and -D) are detected with H-2K^d-, H-2D^d-, and H-2L^d-specific monoclonal antibodies. Additional evidence that MuLV infection alters the expression of normal class I proteins is provided by our findings, using class I-specific CTL, that the immunological function of these antigens parallels their cell surface levels as assessed by monoclonal antibodies.

We show here an increase in the steady-state levels of both H-2 and beta-2 microglobulin mRNA species in MuLV-infected cells, and, in other studies, have demonstrated a parallel increase in transcription of these genes, as assessed by nuclear run-off assay [30]. Therefore, the increased level of H-2 protein in MuLV-infected cells is most likely the result of an increase in the rate of transcription of the H-2, as well as the beta-2 microglobulin, genes. Having observed parallel regulation of three class I genes which are widely separated within the mouse MHC locus, as well as the gene encoding beta-2 microglobulin, which is genetically unlinked to MHC and lies on a separate chromosome, it was of interest to determine whether MuLV infection

would satisfy other criteria for a *trans*-acting mechanism of transcriptional activation. Accordingly, we have shown here that MuLV infection of a heterogeneous population of cells transfected with a human class I MHC gene (HLA-A2) results in enhanced expression of that A2 antigen. The incorporation of exogeneous genes into the cellular genome after DNA-mediated gene transfer is known to be a random process [31]. It is therefore improbable that this induction of MHC antigen expression is the result of a *cis*-acting effect of the integrated provirus on transcription of each of the endogenous class I genes, the beta-2 microglobulin genes, and each of the randomly integrated HLA-A2 genes which were introduced by transfection.

A *trans*-activating effect by MuLV should also be capable of enhancing transcription of unintegrated class I genes or chimeric genes consisting of class I upstream elements linked to a reporter gene in a transient expression assay. Hybrid K^b-CAT constructions were utilized to test for such an activity and to provide some information relating to the genomic control sequences which respond to this regulatory activity. The temporal constraints on transient expression assays allow for minimal, if any, association of the transfected CAT vectors with the cellular genome, and most of the CAT mRNA is derived, therefore, from transcription of the vectors as extrachromosomal elements. The expression of CAT directed from at least 1.2 kb of H-$2K^b$ upstream sequences, when transfected into M-MuLV-infected cells, mimicked the MuLV-induced up-regulation of expression of the whole H-$2K^b$ gene.

Molecular mechanisms other than *trans*-regulation have been proposed to explain the effects of certain retroviruses on H-2 protein expression. Merulo et al. [32] have obtained evidence for altered methylation and structural rearrangements of the MHC complex in Radiation Leukemia Virus (RadLV)-induced tumors, presumably because of proximal integration of the viral genome. Like MuLV, RadLV induces class I MHC expression soon after infection; however, RadLV tumors that arise after a long latent period express little or no H-2 antigen [33].

Interferons are capable of inducing class I MHC expression on mesenchymal cells, and endogenous cellular interferon beta can be elaborated by cells in response to infection with some viruses. Although the response of cells to interferon gamma and to M-MuLV infection is similar [11], we have ruled out interferon as the mediator of MuLV induction of MHC in other studies [30]. We demonstrated that M-MuLV-infected cells do not produce detectable levels of interferon, or any other H-2-enhancing soluble factor, express no detectable mRNA species for interferon beta, and exhibit no interferon-induced antiviral state. In addition, we have previously found that interferon treatment of M-MuLV-infected cells has an additive effect on the H-2 level, suggesting that the site of action of interferon differs from that of the virus. Friedman and Stark have identified a consensus sequence upstream of the protein-coding regions of certain interferon-regulated genes [34]. This interferon response sequence lies approximately -137 to -165 bp upstream relative to the cap site in H-$2K^b$ [27]. Thus the interferon target sequence, as well as the presumably distinct target sequence(s) for MuLV control, are within the 1.2 kb of upstream H-$2K^b$ DNA contained in our chimeric CAT constructions. Fine mapping of these control regions is under way, to determine if they are truly discrete.

The murine sarcoma viruses have evolved a mechanism for keeping MHC expression in the cells they infect to a level suboptimal for recognition and killing by virus-specific or tumor-specific cytotoxic T lymphocytes [11]. Why the leukemia viruses would encode an activity designed to enhance the levels of these same proteins

is not as immediately understandable. Other investigators have demonstrated an association between enhanced or activated expression of certain class I MHC antigens on solid tumors and increased potential for metastasis and invasion [13; and see 28 and 29 for review]. It was postulated that the association of tumor antigens and these enhanced class I antigens might activate a suppressor lymphocyte network, thus inhibiting the immune destruction of the tumor. Alternative hypotheses are possible to provide a teleologic explanation for MHC enhancement by the leukemia viruses. MuLV, and other murine retroviruses such as RadLV, promote the induction of thymic leukemias only after a long latent period through a complex multistep process, involving the generation of novel recombinant retroviruses [35]. After infection by these thymotropic retroviruses, and during the preleukemic phase, high levels of class I MHC antigens and viral gene products are detected on the majority of cells in the thymus [36,33]. It has been proposed that the increased levels of these cell surface proteins may help to bring about the chronic immunostimulation which appears necessary during this stage for eventual leukemogenesis, either to provide the recombinant leukemogenic viruses with a proliferating target cell population [37,38], or perhaps to enhance autostimulation of MuLV-infected, MuLV-specific T lymphocytes, producing a premalignant lymphoid hyperplasia [39]. Thus, the ability of MuLV to enhance H-2 expression in its target cells may be intrinsic to its ability to eventually generate lymphoid neoplasia.

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