

ALTERED TRANSCRIPTION OF GENES CODING FOR CLASS I HISTOCOMPATIBILITY ANTIGENS IN MURINE TUMOR CELLS*

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Expression of cell surface antigens such as viral proteins, differentiation markers (TL, Lyt), and tumor-specific transplantation antigens (TSTA), has been intensively studied on murine tumor cells (for reviews, see references 1–3). Abnormal expression of the major class I histocompatibility antigens (in brief, H-2 antigens in the mouse) has often been observed in spontaneous and virio- or chimio-induced tumors (4–6). In certain instances, expression of new H-2 specificities has been reported (7–9). In others, there was no change in serological specificities, but quantitative differences in the expression of the products of the H-2D and H-2K loci were detected—sometimes an increase in expression but generally either a decrease of both K and D products, or a selective decrease of K alone (5, 6, 10). Such anomalies have most often been described in hematopoietic (lymphoid and erythroblastic) tumor cells, but have also been reported in solid tumors induced by methylcholanthrene (11). Decreased H-2 expression is particularly frequent in leukemias appearing in AKR mice and erythroblastoses induced by Friend virus. It has been suggested that in some cases the decrease in H-2 antigen at the cell surface could be due to antigen masking at the cell membrane (12). H-2 heavy chains could also be present in the cytoplasm and not be exported to the surface, as happens, by lack of active β 2-microglobulin (β 2-m), in human Daudi cells (13, 14). There has also been reported a loss of H-2 expression in cells that have been maintained for many generations in vitro (15, 16). This in vitro modulation of H-2 expression is reversible and after one passage in vivo, the H-2 antigens can be redetected at the cell surface.

Cloned molecular probes corresponding to H-2 and β 2-m gene sequences have become available in this (17–19) and other laboratories (for review, see reference 20). H-2 and β 2-m genes and their transcripts can thus be analyzed in these pathological situations, a study that has not been carried out before. We have shown earlier that some tumors induced by Moloney murine leukemia virus (M-

* Supported by grants from C.N.R.S. (E. R. 201 and A. T.P. 5039), I.N.S.E.R.M. (S. C. 20 and P. R. C. 124031) and the Fondation pour la Recherche Médicale Française.

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MLV) have an altered H-2 expression (21). We selected a number of such tumors and demonstrate here that in at least some of these tumors, abnormal expression is related to a reduced amount of H-2 mRNA. In one case, an important decrease in the quantity of H-2 mRNA is correlated with the absence of detectable cell surface antigens coded by several H-2 genes and suggests a common regulatory defect, perhaps situated at the transcriptional level.

Materials and Methods

Cell Lines. B4R, BM4R, and BM5R were initially obtained as primary tumors following injection of Moloney murine leukemia virus (M-MLV) into mice with a B10 background (see Table I).

Primary tumors were established as continuous cell lines in vitro and maintained in RPMI 1640 Dutch modification (Flow Laboratories) supplemented with 10% heat-inactivated fetal calf serum, antibiotics, and 5×10^{-5} M β -mercaptoethanol. They were frozen in liquid nitrogen after ~ 1 mo of in vitro passaging. The H-2 typing and RNA extractions were done at 2–3 mo. In vivo transplantation of these cell lines was regularly obtained by treating syngeneic mice with pristane (2,6,10,14-tetramethyl pentadecane), 0.5 ml intraperitoneally 10 d before intraperitoneal inoculation of 10^7 cells. Sublethal irradiation (400 R) was performed 3 d before the cell challenge. In these conditions, all the inoculated mice developed either ascitic, mesenteric, or splenic tumors within 3 wk.

LSTRA and MBL2 were initially obtained as primary M-MLV-induced tumors in BALB/c and C57BL/6 mice respectively (27). They were maintained as ascitic tumors by serial in vivo transplantation in syngeneic animals. These two cell lines are routinely used by several laboratories as target cells for T cell-mediated cytotoxicity studies following inoculation of Moloney murine sarcoma virus. They were, therefore, used as H-2-positive control lymphoid cells.

H-2 Typing. H-2 antigen expression on cell lines was initially studied by microcytotoxicity assay using conventional H-2 antisera, (reagents 1–4 in Table II). The monoclonal antibodies 20-8-4S, 28-14-8S, 11-4-1, and 34-2-12 were kindly provided by D. Sachs and K. Ozato (National Cancer Institute) (23–25). These antibodies were also used to confirm the H-2 antigen expression by immunofluorescence. The H-2^f expression was verified by absorption of the antiserum at one dilution above the 50% cytotoxic value on B10M splenocytes. Absorbed antisera were retested on B10M lymphoid cells. The anti-TL antiserum was kindly provided by F. W. Shen (Memorial Sloan-Kettering Cancer Center, New York).

DNA and RNA Extractions. DNA was extracted from mouse tissues or cell lines as described by Gross-Bellard (26). RNA was extracted with lithium chloride and urea, according to Auffray and Rougeon (27). The polyA⁺ mRNA was purified on oligo-dT cellulose columns (19).

"Southern" and "Northern" Blottings. The high molecular weight DNA was cleaved with different restriction enzymes in conditions recommended by the manufacturer (New

TABLE I
Mouse Strains and Derived Tumors Cell Lines

Strain		Tumor
BALB/c	K ^d D ^d	LSTRA
C57Bl/6	K ^b D ^b	MBL2
B10.A(4R)	K ^b D ^b	B4R
F ₁ B10M \times B10.A(4R) = B10M \times 4R	K ^b D ^b /K ^f D ^f	BM4R
F ₁ B10M \times B10.A(5R) = B10M \times 5R	K ^b D ^d /K ^f D ^f	BM5R

TABLE II
Serological Reagents

Reagents	Antibody specificity	Donor lymphoid cells	Recipient strain
<i>Alloantisera</i>			
1	α H-2 ^d	BALB/c	(C3H \times BALB.B)F1
2	α H-2 ^k	BALB.K	(B6 \times BALB/c)F1
3	α H-2 ^b	BALB.B	(C3H \times BALB/c)F1
4	α H-2 ^f	B10M	B10.W.B.
5	α H-2 ^b	B10	B10.BR
6	α TL, 1, 2, 3, 5 & Qa-1	ASL1	(B6 \times A.Tla ^b)F1
7	α Thy-1-2	Thymus C3H	AKR
<i>Monoclonal antibodies</i>			
8 = 20-8-4S	α K ^b , D ^b , K ^d , r, s	C3H-SW	C3H
9 = 28-14-8S	α D ^b , L ^d , q	C3H-SW	C3H
10 = 34-2-12	α D ^d	BDF1	C3H
11 = 11-4-1	α K ^k , K ^q , p, r	CKB	BALB/c

England Biolabs, Beverly, MA). 20 μ g of cleaved DNA per slot was subjected to agarose gel electrophoresis and then transferred to nitrocellulose paper, as described (28). Northern blots were carried out according to Thomas et al. (29), as described previously (19). Relative amounts of H-2 RNA sequences were estimated in a dot blot assay by directly applying 400 ng to 4 ng of poly A⁺ RNA or 40 μ g to 0.4 μ g of total RNA to the nitrocellulose filter. The filter was hybridized to the H-2 probe in the same conditions as for the "Northern" blots.

Results

Serological Analysis of Tumor Cells. We have selected for analysis three thymomas, named B4R, BM4R, and BM5R, isolated as described in Material and Methods and deficient in the expression of H-2 antigens at the K and/or D locus. B4R, BM4R, and BM5R were isolated to B10 recombinant genetic backgrounds, homozygous (B4R) or heterozygous (BM4R and BM5R) at the H-2 region (Table I). For comparison, we used two other lymphomas, also induced by M-MLV, named LSTRA and MBL2, which are known to express the products of both H-2 regions (22) (E. Gommard, personal communication).

For serological typing, B4R, BM4R, and BM5R cells were grown in culture from the primary tumor for no more than 8–12 wk and analyzed by complement-mediated cytotoxicity and immunofluorescence, using reagents listed in Table II. In most cases, these results were confirmed by absorption of specific sera on tumor cells, splenocytes, and thymocytes and retested on target splenocyte cells. (Some tests were also performed on the tumors grown in vivo). Results are summarized in Table III.

The H-2⁺ tumor cells taken as controls, LSTRA and MBL2, were respectively negative and positive for Thy-1-2. B4R, BM4R and BM5R were Thy-1-2 positive, in agreement with their thymic origin. However, B4R, BM4R, and BM5R have abnormal H-2 expression: B4R (from a homozygous background H-2K^kD^b) does not express K^k but retains expression of D^b; BM4R (from a heterozygous background H-2K^kD^b/H-2^f) has lost expression of K^k and D^b while retaining H-

2^f expression; BM5R (from a H-2K^bD^d/H-2^f heterozygous background) is negative for H-2K^b and H-2D^d and H-2^f. Absorption experiments were especially necessary with the anti-H-2^f serum. As the immunizing lymphoid cells from B10M mice are known to produce spontaneously endogenous MLV, the serum may contain antiviral activity. In fact, BM4R and BM5R were both positive by direct cytotoxicity. However, absorption experiments showed that BM4R was H-2^f-positive while BM5R was H-2^f-negative.

The nonexpression of the H-2 regions for the three tumors was verified by measuring their immunofluorescence with different antisera in a fluorescence-activated cell sorter (Cytofluorograph 50HH, Microprocessor MP 2000; Ortho Diagnostic Systems, Westwood, MA) (see Table III). Background values obtained confirmed that B4R is K^k-/D^{b+}, BM4R is K^k-/D^{b-}, and BM5R is K^b-/D^{d-}. Therefore, it seemed to us that B4R, BM4R, and BM5R were good candidates for representing cell lines with altered H-2 expression: at the H-2K locus of both chromosomes (B4R); at the H-2K and H-2D loci of one chromosome, while retaining expression on the other (BM4R); at the H-2 regions of both chromosomes (BM5R). Particular attention was paid to the latter tumor, which was analyzed upon both in vivo growth in the animal and in vitro culture of tumor cells. Serological analysis on both the in vivo and in vitro propagated tumor did not indicate any modulation in H-2 expression related to the mode of propagation and this observation was further assessed by the analysis of genes and transcripts.

Analysis of H-2 Genes and of the β 2-m Gene. For determining whether H-2 and/or β 2-m genes have been deleted in tumor cells, or rearranged in any way that could account for abnormal expression, we analyzed tumor DNA by Southern's technique (28).

The H-2 probe used was the 610 bp long *Hinf*I-*Hinf*I fragment of plasmid pH-2^d-4 (18). It corresponds to a portion of an H-2 cDNA coding for the third

TABLE III
Serological Analysis of Tumor Cells.
Method of Detection of H-2 Determinants.

	Complement-mediated cytotoxicity	Absorption of serum	Immunofluo- rescence	FACS
B4R	H-2 ^b + 3	NT	+ 9	+ 9
K ^k -D ^{b+}	H-2 ^k - 2*	- 2*	- 11	- 11
	Thy-1-2 + 7	NT	NT	NT
	TL + 6	NT	NT	NT
BM4R	H-2 ^b - 3	- 5	- 9	- 9
K ^k -D ^{b-}	H-2 ^k - 2	NT	- 11	- 11
H-2 ^{f+}	H-2 ^f + 4	+ 4	+ 4	NT
	Thy-1-2 + 7	NT	NT	NT
	TL + 6	NT	NT	NT
BM5R	H-2 ^b - 3	- 5	- 8	- 8
K ^b -D ^{d-}	H-2 ^d - 1	- 1	- 10	- 10
H-2 ^{f-}	H-2 ^f + 4	- 4	- 4	NT
	Thy-1-2 + 7	NT	NT	NT
	TL + 6	NT	NT	NT

* Tests were performed on cells grown in vivo and in vitro.
NT, not tested.

extracellular domain, transmembrane and cytoplasmic regions of H-2 heavy chains. It has been shown earlier that this probe (or probes similar to it) detects many restriction fragments in the mouse genome, in relation to the fact that H-2 genes belong to a relatively large multigene family, which comprises at least 36 members in BALB/c mice (30-32). The β 2-m probe was the 685 bp long *HhaI-HhaI* fragment of plasmid p β 2-m1 (19). In contrast with the H-2 probe, the β 2-m probe reacts with only a few fragments in the mouse genome, the interpretation of data being consistent with the existence of a unique β 2-m gene (33). For the Southern analysis, DNA from each type of tumor was compared with DNA of the parent mouse, using several restriction enzymes.

Fig. 1, A shows *EcoRI* digests of DNA extracted from BALB/c liver and LSTRA (lanes a and b) and B10.A(4R) and B4R (lanes c and d, respectively), hybridized with the H-2 probe. Fig. 1, B shows *BamHI* digests of DNA from C57BL/6 liver and MBL2 (lanes a and b) as well as B10M \times 5R (liver and thymus and BM5R tumor cells (lanes c, d, and e, respectively). Fig. 2, A shows *MspI* digests from BioMX5R liver and thymus (lanes a and b), BM5R grown in vivo (mesenteric lymph node) (lane c) or in vitro (lane d), as well as B10M \times 4R liver (lane e) and BM4R tumor DNA (lane f). Again, BALB/c (liver and thymus) and LSTRA DNA, as well as C57BL/6 (liver and thymus) and MBL2 DNA were used as controls (Fig. 2, B, lanes a, b, c, d, and e, respectively). In all cases, patterns of hybridization of tumor DNAs were of similar complexity to those of the parent animals and no difference could be detected.

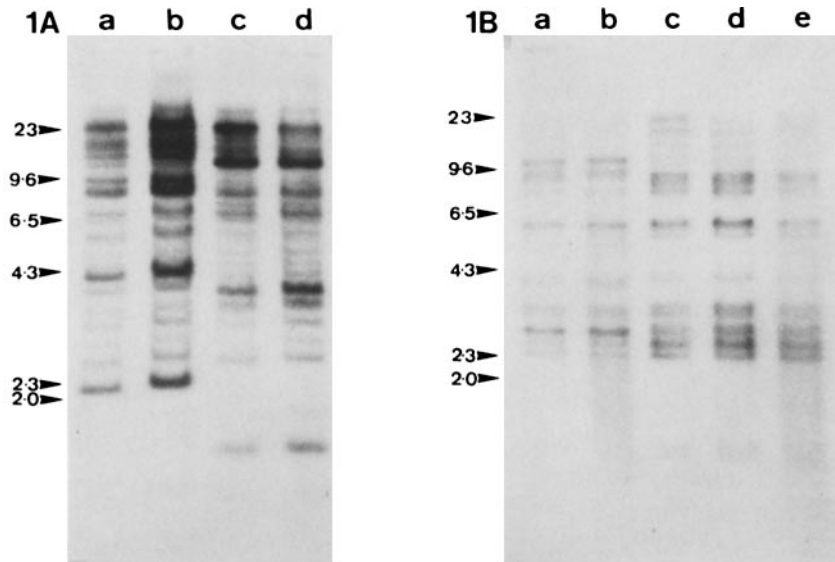


FIGURE 1. Southern blot analysis of H-2 genes with *EcoRI* and *BamHI*. (A) *EcoRI* digested DNAs from BALB/c liver, LSTRA, B10.A(4R) liver, and B4R mesenteric lymph node (lanes a, b, c, and d, respectively). The samples (20 μ g) were run in 0.7% horizontal agarose gels, transferred to nitrocellulose filters, and hybridized to 50 ng of nick translated H-2 probe (specific activity about $2 \cdot 10^8$ cpm/ μ g). Sizes of DNA marker fragments (λ DNA digested with *HindIII*) are indicated in kilobase pairs (kb). (B) *BamHI* digested DNAs from C57BL/6 liver, MBL2, B10M \times 5R liver, B10M \times 5R thymus and BM5R mesenteric lymph nodes (lanes a, b, c, d, and e, respectively), hybridized to H-2 probe.

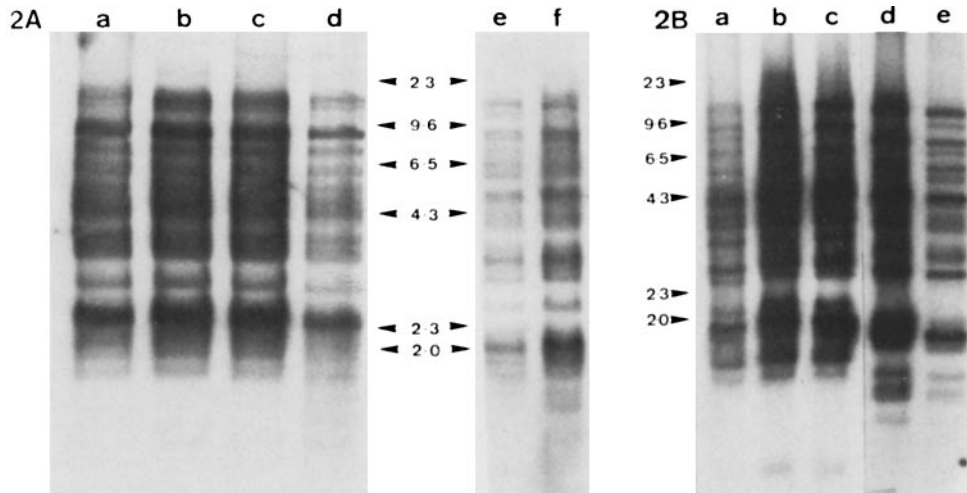


FIGURE 2. Southern blot analysis of H-2 genes with *MspI*. (A) *MspI* digests of DNA from B10 \times 5R liver, thymus, BM5R mesenteric lymph nodes, BM5R cells grown in vitro, B10M \times 4R liver, BM4R ascitic tumor (lanes a, b, c, d, e, and f, respectively), hybridized to H-2 probe as described in Fig. 1. (B) *MspI* digests of DNA from BALB/c liver, thymus, LSTRA, C57BL/6 liver, and MBL2 (lanes a–e) hybridized to H-2 probe.

We examined more closely, in two respects, the DNA of the seemingly H-2-negative tumor BM5R. First, we analyzed the state of methylation of H-2 genes using *MspI* and *HpaII* restriction enzymes. These enzymes restrict DNA at the same site, except that *HpaII* does not cleave when the CpG in the site is methylated (34–36). Results in Fig. 3, A reveal no drastic difference between liver DNA from the parent animal and the DNA of BM5R grown in vivo or in vitro (digested with *EcoRI* and *MspI*, lanes a, c, and e; digested with *EcoRI* and *HpaII*, lanes b, d, and f). Second, we looked for the β 2-m gene with the β 2-m probe. Results in Fig. 3, B show the presence of the characteristic 2.8-kb and 6-kb *EcoRI* bands (33) in B10M \times 5R liver and thymus (lanes a and b) and BM5R DNA (lane c). Similar results were obtained with other tumors (data not shown).

In summary, these experiments have detected no structural change, either in the H-2 multigene family or in the β 2-m gene, that could account for the altered expression of H-2 in tumor cells.

Transcription of the β 2-m and H-2 Genes in Tumor Cells. Transcription of the β 2-m and H-2 genes was assayed with the same probes as above, using the Northern hybridization technique (29). The H-2 probe is known to detect a major RNA band, corresponding to molecules of \sim 1,800 nucleotides, which contains the transcripts of class I genes. Although this H-2 probe probably detects transcripts of all the class I genes, K, D, L, Qa, and TL, the majority of the transcripts that hybridize are from the K and D regions as the analysis of a cDNA bank, made in the laboratory from DBA/2 liver mRNA, has shown that 30% of the clones positive with this probe were K^d transcripts (J. L. Lalanne, personal communication). This probe can therefore be used to monitor predominantly the transcripts of the regions K and D.

The β 2-m probe detects two RNA species of about 800 and 1,000 nucleotides

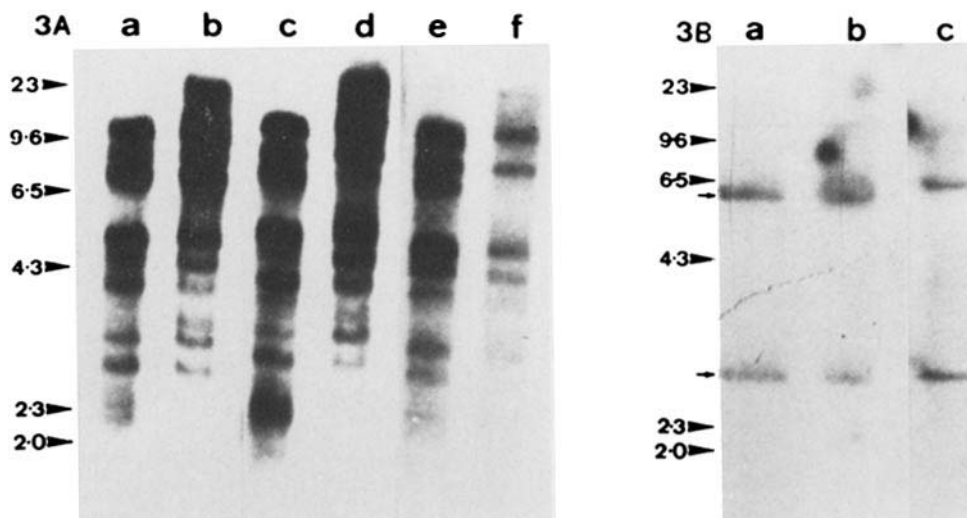


FIGURE 3. Southern blot analysis of BM5R DNA for H-2 genes and the $\beta 2$ -m genes. (A) DNAs from B10 \times 5R liver, BM5R mesenteric lymph nodes and BM5R cells grown in vitro were digested to completion with *EcoRI* plus *MspI* (lanes a, c, e) or *EcoRI* plus *HpaII* (lanes b, d, f), electrophoresed through 0.7% agarose gels, transferred onto nitrocellulose filters and hybridized to H-2 probe, as described under Fig. 1. (B) *EcoRI* digests of DNA from B10M \times 5R liver, thymus and BM5R mesenteric lymph nodes, hybridized to the $\beta 2$ -m probe. The arrows indicate the characteristic fragments of 2.8 kb and 6 kb of the $\beta 2$ -m gene.

(19, 37) that are due to the presence of two polyadenylation sites in the $\beta 2$ -m gene (38).

In the experiments shown in Fig. 4, the two probes were mixed and hybridized to poly A⁺ RNA transferred onto a nitrocellulose filter. C57BL/6 and BALB/c liver RNA display the expected pattern (Fig. 4, A, lanes a and c). MBL2 and LSTRA RNA show the expected bands plus additional material of high molecular weight (Fig. 4, A, lanes b and d). Hybridization with the H-2 probe alone shows that these bands of high molecular weight, as well as a band of RNA of $\sim 1,500$ nucleotides react with the H-2 probe and not the $\beta 2$ -m probe (Fig. 4, B). Results obtained with B4R and BM5R and a mixture of the H-2 and $\beta 2$ -m probes are shown in Fig. 4, C (lanes b and d) together with control liver RNA from C57BL/6 and B10M \times 5R (lanes a and c). These tumor cells have reduced amounts of H-2 RNA, barely detectable in BM5R (lane d). BM5R, however, does make significant amounts of $\beta 2$ -m RNA, as seen in lane h, which shows a longer exposure of lane d. The experiment shows a decrease in the $\beta 2$ -m RNA content, but less drastic than for H-2 mRNA. One should note, however that only the 1,000 nucleotides mRNA is detectable in this tumor although the mRNA from the liver shows the two $\beta 2$ -m bands.

These results could be due to a particularly active degradation of mRNA in tumor cells. The integrity of RNA preparations was assessed in two ways: (a) their ability to direct protein synthesis in vitro in a reticulocyte lysate system (data not shown); (b) hybridization with other probes in the same Northern blots, such as actin, $\beta 2$ -m and retroviral LTR probes (see Fig. 4, D, lane b for hybridization of BM5R in vitro RNA with an SFFV LTR probe; details are given

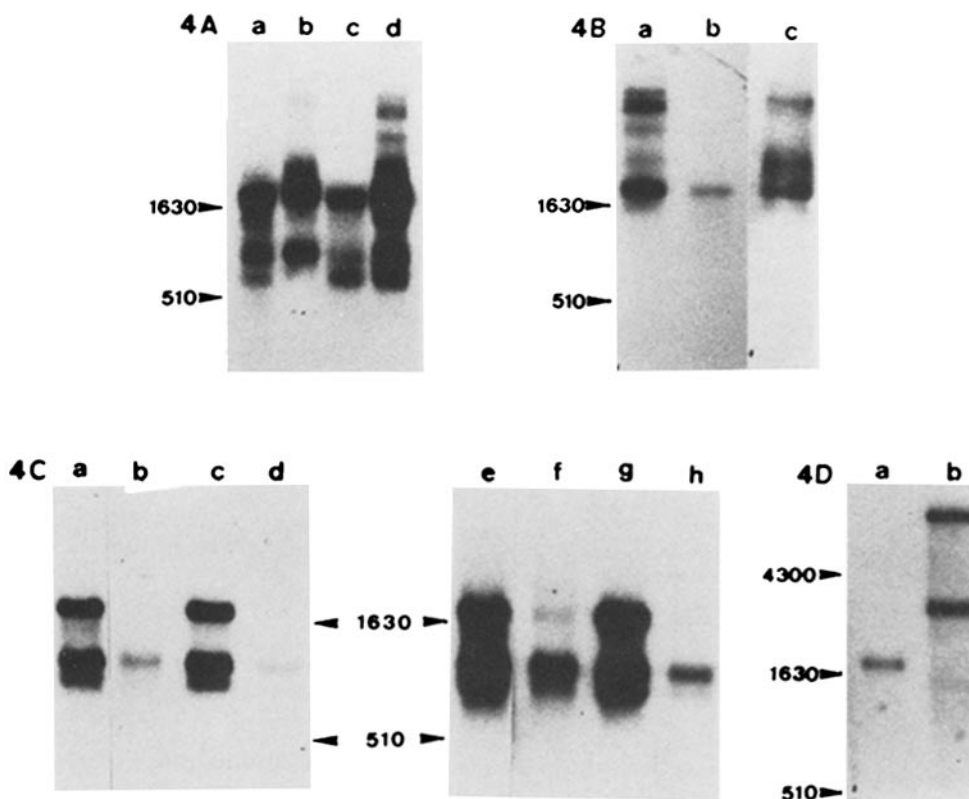


FIGURE 4. Analysis of H-2 and β 2-m transcripts in normal tissues and M-MLV-induced tumors. (A) 5 μ g of poly A⁺RNA were treated as described in Materials and Methods and electrophoresed through 1.1% agarose gels, transferred onto nitrocellulose filters and hybridized to the H-2 and β 2-m probes. Lanes a, b, c, and d contain, respectively, poly A⁺ RNAs from C57BL/6 liver, MBL2, BALB/c liver, and LSTRA. (B) Poly A⁺ RNAs from LSTRA (lane a), BALB/c liver (lane b) and MBL2 (lane c) hybridized to the H-2 probe only. (C) Poly A⁺ RNAs from C57BL/6 liver, B4R ascitic tumor, B10M \times 5R liver, and BM5R cells grown in vitro (lanes a, b, c, and d, respectively) hybridized with a mixture of the H-2 and β 2-m probes. Lanes e, f, g, and h correspond to a longer exposure of lanes a, b, c, and d, respectively. (D) Poly A⁺ RNAs from B10 \times 5R liver (lane a) and BM5R cells grown in vitro (lane b) hybridized to an LTR probe of SFFV. The LTR probe was a subclone derived from the SFFV genome initially cloned by Linemeyer et al. (39). The SFFV plasmid was digested to completion with *Eco*RI and *Pst*I and the 1.8 kb *Eco*-*Pst* containing the LTR was ligated to pBR327 digested with *Eco*-*Pst* (B. Cami, personal communication).

in the legend). These controls showed that the RNA preparations used were intact and that degradation, unless extremely selective for H-2 mRNAs, could not explain the very much reduced amounts of H-2 mRNA in BM5R.

To evaluate the quantity of the H-2 RNA made in the different tumor cells, we carried out a dot blot hybridization with a series of semi-logarithmic dilutions of each sample of RNA (poly A⁺ or total) spotted on the filter. Comparison of the intensities of the spots on the autoradiograph (confirmed by densitometer scanning) permits a semi-quantitative estimate of the relative amounts of H-2-specific RNA in poly A⁺ RNA (Fig. 5). Salient features are that: (a) LSTRA (row 8) makes several times more H-2 RNA (3–10 times) than spleen (row 3); (b)

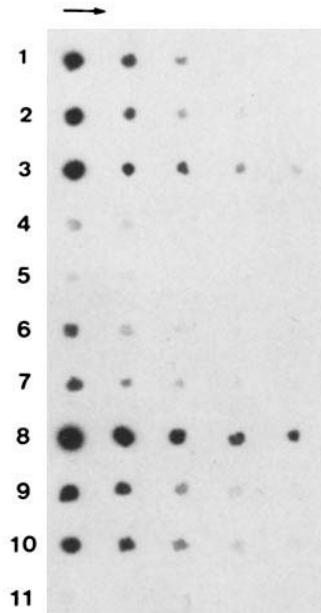


FIGURE 5. Estimation of the relative H-2 mRNA abundance in normal tissues and M-MLV-induced tumors by dot blot analysis. Decreasing amounts of poly A⁺ RNA (400 ng–4 ng) or total RNA (40 μ g–0.4 μ g) were applied to the nitrocellulose filter and hybridized to the H-2 probe as described in Materials and Methods. Poly A⁺ RNA from C57BL/6 liver, thymus, or spleen (rows 1, 2, and 3, respectively), BM5R cells grown in vitro or in vivo (rows 4 and 5), BM4R cells grown in vitro (row 6), B4R grown in vivo (row 7), LSTRA and MBL2 (rows 8 and 9, respectively). Total RNA from C57BL/6 thymus (row 10) and BM5R cells grown in vitro (row 11).

BM5R grown in vitro or in vivo (rows 4 and 5) makes 10–30 times less RNA than liver or thymus (rows 1 and 2) and 30–100 times less than spleen (row 3); (c) BM4R and B4R (rows 6 and 7) yield intermediates levels; (d) there is no detectable accumulation of H-2 sequences in the poly A⁻ RNA of BM5R since total RNA of this tumor yields very weak signals (row 11) as compared with total thymus RNA of C57BL/6 (row 10).

Discussion

We have analyzed three tumor cell lines that display deficient H-2 expression of either one gene on a homozygous background (B4R) or several H-2 genes from one or both chromosomes in a heterozygous background (BM4R and BM5R). Tumor DNA and RNA were analyzed with cloned H-2 and β 2-m cDNA probes using the Southern and Northern techniques.

DNA analysis reveals no observable difference between genes of the tumors and of the parent animals. It must be noted, however, that the Southern pattern of H-2 genes is complex, and that the probe used was characteristic of the 3' half of H-2 genes and did not detect the 5' half. These experiments, therefore, suggest strongly that there is no gross deletion or rearrangement of H-2 genes, but we cannot rule out deletion of one gene and/or rearrangement at 5' ends or some minor lesions that affect the expression of the H-2 genes.

Analysis of RNA showed a decrease in mRNA hybridizing to the H-2 probe in these cells correlated with abnormal H-2 expression. Particular attention was given to BM5R for which serological data suggested an H-2-negative phenotype. H-2 mRNA in BM5R was very much reduced, while $\beta 2$ -m RNA, albeit diminished, was easily detected. Lack of $\beta 2$ -m is, therefore, not the origin of the H-2-negative phenotype, which is more likely to be due to decreased transcription of the H-2 genes. The weak signal of hybridization (a few per cent of control) could be due either to H-2 expression in a small percentage of the cell population, or to cross-hybridization of the H-2 probe with H-2-like sequences such as those coding for T1 or Qa-1 antigens (20, 40, 41). Preliminary serological data suggest that BM5R expresses TL antigen, so that we rather favor the latter hypothesis. In vitro modulation of H-2 expression in BM5R was ruled out by the analysis of DNA and RNA in tumor cells grown in vivo and in vitro.

Decrease in the expression of H-2 antigens at the surface of tumor cells could make them insensitive to H-2-dependent and H-2-restricted cellular cytotoxic reactions and be one of several means favoring tumor progression. In this regard, growth of tumors in an animal could be a way of isolating or selecting variant or mutant cells with altered H-2 expression. Our studies with different combinations in the loss of H-2 expression (one locus, both loci of one chromosome, loci of both chromosomes) give no clue as to a possible common mechanism inactivating one or several H-2 genes. In particular, all tumors were induced by M-MLV and our present studies have not revealed viral integration in the immediate vicinity of H-2 genes which would have altered the Southern patterns (data not shown).

In the first combination (H-2K⁻D⁺), there exist somatic cells with the same H-2 phenotype in a compartment of the thymus (42). B4R tumor cells could, therefore, derive from the latter. In the other two combinations, there is no known somatic equivalent. Embryonic cells express neither H-2 heavy chains nor $\beta 2$ -m and neither of these genes is transcribed in embryonal carcinoma cells (19, 37). The situation in BM5R is different, since it expresses significant amounts of $\beta 2$ -m RNA, but it cannot be ruled out that some embryonic cells have the same behavior. The fact, however, that BM4R and BM5R are thymomas strongly suggests that these tumors have acquired their H-2-negative character by some kind of alteration in the regulation of H-2 genes rather than representing some immortalized intermediate stage of cell differentiation. Our results would then point to the existence of a mechanism(s) that coordinately regulates the expression of distant H-2 genes on one or both chromosomes. Human cell lines negative for the expression of class I HLA antigens have been selected in vitro by Gladstone et al. (43) with similar conclusions (see also reference 44). These various tumor cell lines could thus provide a useful material to analyze further this hypothetical regulation of H-2 gene expression.

Summary

Three murine tumors induced by Moloney murine leukemia virus (M-MLV) which exhibited loss of some or all H-2 class I antigens at the cell surface were analyzed at the DNA and RNA level with molecular probes specific of H-2 heavy chains and $\beta 2$ -microglobulin sequences. No observable difference could be detected at the DNA level between the tumors and the parent animals. However,

a decrease in H-2 mRNA was observed, especially in phenotypically H-2 negative tumor, BM5R, where H-2 transcripts were at least 30-fold less abundant. These results show that an H-2-negative character may result from a general alteration in the transcription of H-2 genes, which could reflect some kind of regulatory process.

We are grateful to Drs. S. Sachs, M. Pierres, for their generous gift of monoclonal antibodies, F. W. Shen for anti-TL-antibody and to Dr. E. M. Scolnick for providing the cloned SFFV genome. We also thank Dr. Metezeau for use of the Cell Sorter (S.C. I.N.S.E.R.M.-Pasteur "Trieur de Cellules") and Dr. Y. Cayre for help with the serological analysis.

Received for publication 4 April 1983 and in revised form 12 July 1983.

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