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A Transcription Factor Interacting with the Class I Gene Enhancer Is Inactive in Tumorigenic Cell Lines Which Suppress Major Histocompatibility Complex Class I Genes

ULF HENSELING,[1]† WILHELM SCHMIDT,[1] HANS R. SCHÖLER,[2] PETER GRUSS,[2] AND ANTONIS K. HATZOPOULOS[2]‡*

Institut für Zellbiologie (Tumorforschung), Universität Essen (GH), Hufelandstrasse 55, D-4300 Essen I,[1] and Max-Planck-Institut für biophysikalische Chemie, Abteilung Molekulare Zellbiologie, D-3400 Göttingen,[2] Federal Republic of Germany

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AKR leukemias display different amounts of major histocompatibility complex class I antigens on the cell surface. The absence of H-2K^k molecules correlates with the ability of these cell lines to form tumors in vivo as well as to escape lysis by cytotoxic T lymphocytes in vitro. In this report it is shown that the 5′ regulatory area of the *H-2K^k* gene failed to activate transcription in *H-2K^k*-negative cells. Examination of the proteins interacting with the *H-2K^k* enhancer in expressing and nonexpressing cells revealed clear differences. In particular, the level of a nuclear protein interacting at position −166 was greatly reduced in the negative cell lines. A transcription factor, known as H2TF1 or KBF1, has been shown previously to interact with this binding site and to be essential for the expression of certain class I genes as well as the expression of β_2-microglobulin. These results demonstrate that the molecular mechanism of class I gene suppression in malignant tumor cells is at the level of transcription and is most probably modulated by H2TF1/KBFI. In addition, it is shown that the same transcription factor is only present in mouse tissues expressing class I antigens.

The mouse major histocompatibility complex (MHC) is located on chromosome 17 and encodes the highly polymorphic cell surface transplantation antigens H-2K, H-2D, and H-2L (18, 21). These 45- to 48-kilodalton (kDa) glycoproteins are noncovalently associated extracellularly with a nonpolymorphic 12-kDa polypeptide called β_2-microglobulin (β_2m) (9). β_2m, encoded on chromosome 2, is essential for membrane insertion of class I molecules and stabilization of their tertiary structure (1, 28, 39).

Class I antigens are expressed on most murine somatic cells, although in different amounts. There are few or no class I antigens in the central nervous system and pancreas, intermediate levels in liver and kidney, and high levels in spleen, lung, and muscle (25). Similar distribution was also reported in human tissues (10). Class I expression can be stimulated in various cell types by interferons (42). Interestingly, there is no expression of class I antigens in early development and in embryonal carcinoma (EC) cells (37). Class I expression is induced after differentiation of EC cells and corresponds with the appearance of class I antigens later in mouse development (36, 40, 43).

The class I antigens are involved in several pathways of the immune system (21). They play an important role in self-nonself discrimination as specific target antigens to cytotoxic T lymphocytes. Viral antigens on the surface of infected cells and tumor-associated antigens on cells altered by neoplastic transformation are recognized only in the context of MHC class I antigens (19, 63). Tumor cells frequently show quantitative alterations in their expression of H-2 molecules compared with cells from the corresponding normal tissues (11, 16, 46, 52, 57). Reduced expression of one or more H-2 antigens appears to be associated with the ability of tumor cells to escape surveillance by cytotoxic T lymphocytes and in some cases can facilitate metastatic spread (8, 47, 58).

Due to multiple retroviral insertions, ageing AKR mice frequently develop tumors, particularly thymomas, that can easily be grown in cell culture (35). Independently isolated AKR tumor cell lines express variable amounts of H-2K^k molecules on their surface (48). AKR tumor cells with normal or high levels of H-2K^k molecules are readily lysed by cytotoxic T lymphocytes and cannot form tumors when reintroduced into normal AKR mice (14, 47). Cell lines with reduced H-2K^k expression cannot be killed by specifically sensitized T lymphocytes and can form tumors. It appears that the lack of H-2K^k antigens gives the AKR leukemias a selective advantage for tumor progression. Consequently, to understand the molecular basis of tumorigenesis, the mechanism of class I antigen suppression must be elucidated.

After restoration of H-2K^k expression in negative cells by gene transfection, AKR tumors lose their ability to grow in vivo (22). These results, as well as preliminary RNA analysis, indicate that expression of the H-2K^k antigen in AKR leukemias is regulated at the transcriptional level (22, 48). The transcriptional control of MHC class I genes has been extensively studied, and a number of *cis*-acting elements and *trans*-acting factors have been identified. Multiple regulatory sequences contribute to proper expression of the class I genes (6, 26, 27). In particular, a *cis*-acting element found 166 bases upstream of the *H-2K^b* gene is essential for expression. This 5′-flanking regulatory region is highly conserved in the *K^k*, *K^d*, *K^b*, *L^d*, and β_2m genes (26). It shows a

* Corresponding author.
† Present address: Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Carl von Liuné Weg 10, D-5000 Köln 30, Federal Republic of Germany.
‡ Present address: Foundation for Research and Technology HELLAS, Institute of Molecular Biology and Biotechnology, P.O. Box 1527, Heraklion 71110, Crete, Greece.

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perfect dyad symmetry, one part overlapping with a consensus interferon response sequence (23, 26, 27, 36). Two nuclear factors, called H2TF1 and KBF1, interacting with this sequence have been characterized independently (7, 24, 62). At present it is not clear if these proteins are identical or related factors.

Here, it is demonstrated that the levels of *H-2K*^{k} mRNA in AKR leukemia lines reflect the amounts of H-2K^k antigen present in the cell membrane. We cloned part of the 5′ enhancer region of the *H-2K*^{k} gene in front of a reporter gene and show that it functions only in leukemia cells expressing high levels of H-2K^k antigen. Techniques that can probe DNA-protein interactions reveal that H-2K^k-negative cell lines contain significantly reduced levels of a nuclear protein which binds to the highly conserved 5′ regulatory motif. Since this *cis*-acting element is essential for class I gene expression, these results indicate that tumorigenic cell lines suppress expression of class I genes by specifically inactivating the corresponding transcription factor. Moreover, the amounts of this nuclear protein in different tissues vary significantly and parallel the levels of MHC class I antigens present on their surface.

MATERIALS AND METHODS

Cell lines and preparation of nuclear extracts. The AKR (H-2^k) leukemias were kindly provided by P. Krammer (Heidelberg, Federal Republic of Germany [FRG]). They were grown in tissue culture in RPMI 1640 medium, supplemented with 10% heat-inactivated fetal calf serum (FCS), glutamine, antibiotics, and 5×10^{-5} M β-mercaptoethanol.

For extract preparation, 3 liters of cells were grown in glass bottles to a density of 10^6 cells per ml. The extracts were made as described previously (12, 60) and contained between 10 and 20 mg of protein per ml.

Monoclonal antibody. A hybridoma cell line producing H-2K^k-specific monoclonal antibody (MAb 100-5) was obtained from G. Hämmerling (Heidelberg, FRG). The MAb has been characterized (29) and was used as the culture supernatant.

Interferon treatment. Mouse interferon type I (alpha and beta) of a high specific activity (10^8 U/ml) was obtained from A. Senkik (Paris, France). The optimal concentration for the stimulation of *H-2* expression in the AKR leukemic cells was found to be 10^4 U/ml.

Indirect immunofluorescence FACS analysis. Indirect fluorescence staining was performed with 0.01 ml of MAb-containing hybridoma culture supernatant per 5×10^5 cells. A fluorescein isothiocyanate (FITC)-conjugated sheep F(ab′)$_2$ anti-mouse immunoglobulin (New England Nuclear, Dreieich, FRG) was used as the second antibody. Incubation with MAb100-5 was carried out for 1 h at 4°C in 1× phosphate-buffered saline (1×PBS) containing 2% FCS and 0.05% NaN_3 (buffer I). The second incubation with the FITC-conjugated antibody was carried out for 1 h at 4°C in 1×PBS buffer containing 0.1% bovine serum albumin and 0.05% NaN_3 (buffer II). Cells were washed twice in buffer I and resuspended at 10^6 cells per ml for quantitative analysis in a modified fluorescence-activated cell sorter (FACS II; Becton Dickinson, Palo Alto, Calif.) connected to a logarithmic amplifier covering fluorescence intensities over a range of 10^0 to 10^3.

Recombinant plasmids. The following plasmids were used and have been described previously: p209 A21 (17); pH2IIa contains a cDNA which recognizes all class I sequences (56); mouse b_2m cDNA (37); pH-2^d-5b contains a cDNA specific for *H-2K* sequences (61); plasmid pHβAlacZ, which contains the β-actin promoter in front of a β-actin-*lacZ* fusion gene, was kindly provided by A. Püschel (Göttingen, FRG). The cosmid containing the *H-2K*^{k} gene was a generous gift of M. Steinmetz (Basel, Switzerland).

Oligonucleotides. The following oligonucleotides were used for double-stranded DNA for gel retardation: μE1 (5′-ctAgaGTTGAGTTGAGTCAAGATGGCCGATCAGAA CCAGAACACCTGCA); μE2 (5′-GACCGTCCTTCGT CCAGTACACCGTTCCGATAA); H1 (5′-ggg GTCAG GGGTGGGGAAGCCCAGGGCTGGGGATTCCCCA TC); and S10 (5′-cccTGGGGACTTTCCACACCCTA). μE1 and μE2 are the regions 334 to 380 and 385 to 417 of the immunoglobulin heavy-chain gene enhancer (numbering according to reference 13), respectively. Lowercase letters define sequences not present in genomic DNA coding for restriction sites. The H2TF1/KBF1 site in H1 and the NF-κB site in S10 are underlined. For single-stranded DNA for primer extension, the oligonucleotides were U1 (5′-GGTG GTATATCCAGTGATTTTTTTCTCCAT) and P5a (5′-GG CTGGCCGGGCTTACCTGG).

Analysis to mRNA and hybridization. Isolation of total cytoplasmic RNA from AKR leukemias, Northern (RNA) blotting, and hybridization to different cDNA probes were carried out as described previously (48).

Gel retardation. The reaction mix consisted of end-labeled double-stranded oligonucleotides (5,000 to 10,000 cpm), 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), (pH 8.0), 1 mM spermidine trihydrochloride, 5 mM $MgCl_2$, 50 mM KCl, 1 μg of poly[d(I-C)], 8.6% glycerol, and 4 to 8 μg of nuclear extract. The final volume was 15 μl. In the competition experiments, nonradioactive double-stranded oligonucleotides in molar excess were included. The mix was incubated for 15 min at room temperature, and subsequently 1.5 μl of loading buffer (90 mM EDTA [pH 8.0], 0.25% bromphenol blue, 0.25% xylene cyanol, 10% sucrose) was added. The probes were directly loaded onto a native 5% polyacrylamide gel (30:1, acrylamide-bisacrylamide, in 1× TBE [32]). After the run, gels were dried and autoradiographed.

DNase I footprinting. Sequencing reactions and radioactive labeling were carried out according to standard procedures (32). The DNase I footprinting reaction mix consisted of 10 mM HEPES (pH 8.0), 0.1 mM EDTA, 100 mM NaCl, 2 mM dithiothreitol (DTT), 10,000 cpm of end-labeled fragment, 1 μg of poly[d(I-C)] as unspecific competitor DNA, and 40 to 200 mg of nuclear extract. The final volume was 45 μl. For the binding reaction, the probes were incubated for 30 min at 25°C and then transferred on ice. In a next step, 1 μl of 0.1 M $MgCl_2$ and 5 μl (0.5 to 1 μg) of diluted DNase I (Sigma Chemical Co.; stock solution, 2 mg/ml in 50% glycerol) were added. Dilution buffer was 10 mM HEPES (pH 8.0)–0.1 mM EDTA–100 mM NaCl–2 mM DTT. After 5 min at room temperature, the reaction was stopped by addition of 250 μl of 0.2% sodium dodecyl sulfate–0.6 M sodium acetate (pH 5.2)–30 μg of tRNA per ml–300 μl of phenol-chloroform-isoamyl alcohol (25:24:1). After extraction and ethanol precipitation, the pellet was washed in 70% ethanol and suspended in denaturing loading buffer prior to electrophoresis on a denaturing urea–6% polyacrylamide gel (32). For control reactions without protein, the nuclear extract was substituted by dilution buffer and 0.1 to 0.5 μg of DNase I was used.

In vitro transcription. In vitro transcription reactions were carried out in a final volume of 20 μl, with 5 to 10 μl of nuclear extract. Nucleotides were added to give a final

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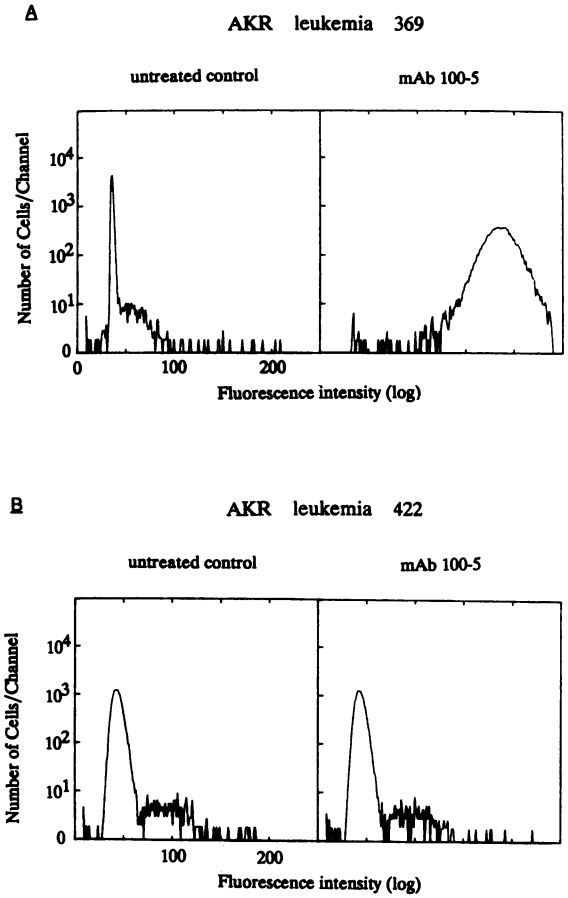

FIG. 1. FACS analysis of leukemia cell lines 369 and 422. The amounts of H-2K^k surface antigen were evaluated by incubating cells first with an anti-H-2K^k monoclonal antibody (MAb 100-5) and subsequently with a sheep F(ab')$_2$ anti-mouse immunoglobulin conjucated to FITC. Quantitative immunofluorescence analysis was carried out with a modified FACS II. (A) 369 cells. (B) 422 cells. The left diagrams represent the fluorescence intensity distribution of control cells incubated with the FITC-labeled antibody alone. The right diagrams show the distribution of cells reacted with both the H-2K^k-specific MAb 100-5 and the FITC-labeled anti-mouse immunoglobulin antibody.

concentration of 0.5 mM each. The DNA concentration was 40 μg/ml. $MgCl_2$ was added to 2 mM, KCl to 50 mM, creatine phosphate to 0.5 mM, and spermidine trihydrochloride to 4 mM. The components were mixed together at room temperature, and the in vitro transcription was carried out for 60 min at 33°C. The reaction was stopped by addition of 250 μl of 0.5 M NaCl–0.1% sodium dodecyl sulfate–1 mM EDTA (pH 8.0)–50 μg of proteinase K per μl–10 mg of tRNA per ml. The samples were extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and ethanol precipitated. The resulting RNA transcripts were quantified by primer extension. As primers, a 30-mer and a 20-mer single-stranded oligonucleotide coding for the first nucleotides of the *cat* gene and part of the β-actin gene sequence were used (oligonucleotides U1 and P5a, respectively; see above for sequences). Then, 50,000 cpm of the end-labeled primers was hybridized to the transcribed RNA overnight at 30°C. After ethanol precipitation, the pellet was carefully washed and dried at room temperature. The primer extension reaction was carried out at 42°C for 1 h in a final volume of 50 μl

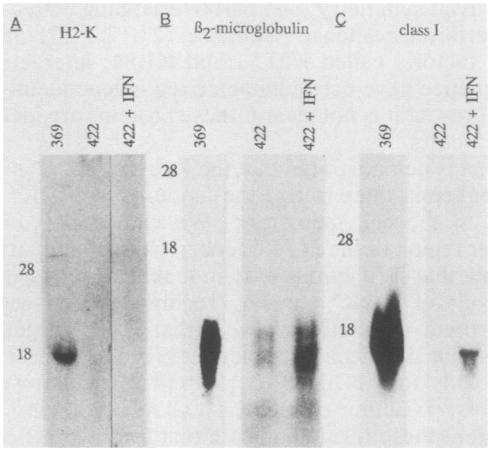

FIG. 2. Northen blot analysis of RNA from cell lines 369 and 422. Total cytoplasmatic RNAs were subjected to electrophoresis in agarose gels, blotted onto Gene-Screen filters, and hybridized with (A) an *H-2K*-specific probe, (B) a β_2m-specific probe, or (C) a probe that detects most class I transcripts. Leukemia cell line 422 was treated with interferon type I at 104 U/ml for 12 h before RNA extraction (lanes 422+IFN). The heading above each lane indicates the RNA source. 28 and 18 indicate the positions of 28S and 18S, respectively.

containing 0.5 mM each of the four dioxynucleotides, 50 mM Tris chloride (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$, 50 mg of actinomycin D per ml, and 100 U of Moloney murine leukemia virus reverse transcriptase. After phenol-chloroform extraction and ethanol precipitation, the pellet was washed with 70% ethanol, suspended in 6 μl of denaturing loading buffer, and applied to a 6% sequencing gel.

RESULTS

AKR leukemias have variable amounts of H-2K^k surface antigens. The quantity of H-2K^k surface antigens in a series of tissue culture-adapted AKR leukemias varies significantly (48). Certain AKR cell lines have very high levels of H-2K^k molecules in their membrane, while others have low or undetectable levels.

In this report, attention was focused on two extreme cases, cell lines 369 and 422. These cells were first incubated with an antibody specific for H-2K^k antigen (MAb 100-5) (29) and subsequently with an affinity-purified FITC-conjugated sheep F(ab')$_2$ anti-mouse immunoglobulin. In the control experiment, the anti-H-2K^k antibody was omitted. After antibody absorbance, the samples were subjected to quantitative FACS analysis. The 369 cells displayed high levels of H-2K^k molecules in their membrane, while 422 cells showed only very low amounts (Fig. 1).

Interferons are known to stimulate class I expression in murine cells (42). The experiment shown in Fig. 1 was repeated after treating the cells with mouse interferon alpha/beta. The pattern obtained in the FACS analysis, however, was similar to that in Fig. 1 (data not shown). It thus appears that interferon cannot induce class I expression in these two cell lines.

Expression of H-2K^k in 369 and 422 cells. Total RNA from positive 369 and negative 422 cells was isolated, and equal amounts were separated in agarose gels. The gels were blotted onto membrane filters and hybridized to either an H-2K^k-specific probe, a β_2m-specific probe, or a probe

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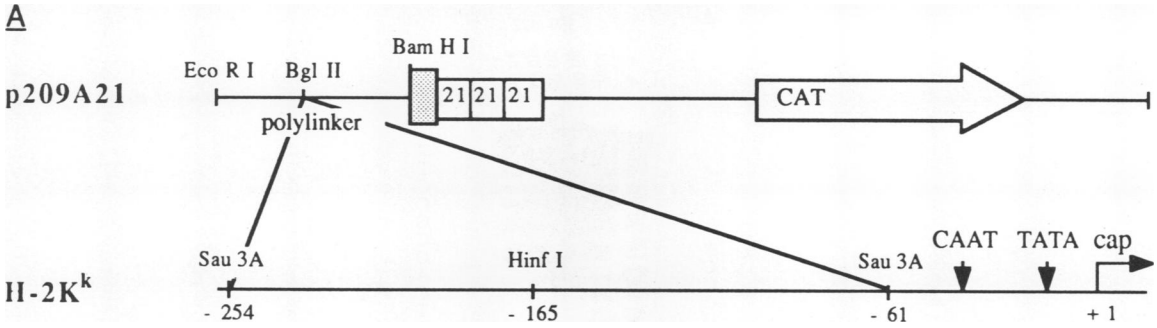

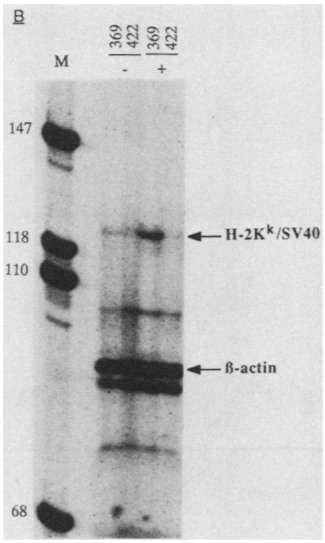

FIG. 3. (A) Schematic diagram of the 5′ region of the *H-2K^k* gene. The 193-base-pair *Sau*3A fragment (2) was cloned in the *Bgl*II site of the polylinker in the p209A21 vector (17). This construct is derived from the pSV2CAT vector (17) and contains the SV40 early promoter in front of the chloramphenicol acetyltransferase gene (CAT). (B) Primer extension of in vitro transcription products. The constructs displayed in panel A were incubated with nuclear extracts prepared from 369 and 422 cells. The RNAs were subjected to primer extension, and the products were evaluated by analysis in denaturing gels. In lanes marked (−), the p209A21 vector was used. In the (+) lanes, the construct with the *H-2K^k* 5′ enhancer region cloned in the *Bgl*II site was used. As an internal control, a β-actin promoter β-actin-*lacZ* gene construct was incubated together with the other plasmids. The arrows designate the accurately initiated transcripts directed by the *H-2K^k* enhancer-SV40 promoter and the β-actin promoter. Lane M, Molecular size markers (in base pairs).

which recognizes all *H-2* class I sequences (Fig. 2). The amount of *H-2K^k* mRNA was significantly reduced in the negative cell line 422 and could not be stimulated by interferon, consistent with the protein results in Fig. 1 (Fig. 2A). Moreover, it appears that β_2m and additional *H-2* class I mRNAs were similarly affected (Fig. 2B and C). Only a slight increase was observed after interferon stimulation.

Southern analysis of chromosomal DNA isolated from positive and negative cells revealed no differences in the *H-2K^k* gene (data not shown). The fact that other independently isolated AKR leukemias show a phenotype similar to that of 422 (48) and the fact that more H-2 antigens and β_2m are suppressed like H-2K^k suggest a general mechanism for inhibition of expression of these genes acting at the transcriptional or an early posttranscriptional level. It is likely that this mechanism affects the transcriptional level, since the promoters of the suppressed genes show similarities in their *cis*-acting transcriptional motifs (26).

The regulatory area of the *H-2K^k* gene fails to activate transcription in non-expressing cells. Part of the 5′ region of the *H-2K^k* gene, which contains the major enhancer (26), was cloned in a plasmid carrying a simian virus 40 (SV40) promoter sequence in front of the procaryotic reporter gene for chloramphenicol acetyltransferase (CAT) (17) (Fig. 3A). In vitro transcription experiments were performed with this construct and nuclear extracts from 369 and 422 cells. As a control, a plasmid carrying a β-actin-*lacZ* fusion gene under the control of the β-actin promoter was used. It has been shown that cell-free systems allow correct RNA polymerase II transcription of exogenous DNA templates (33). Furthermore, it is known that enhancer sequences at the 5′ end of different promoters lead to an in vitro increase in transcriptional activity of exogenous DNA (44, 50, 53, 54). The in vitro transcription was used because the transfection efficiency of the thymoma lines was very low, and therefore electroporation and the DEAE-dextran transfection technique could not be successfully employed in this system, as has been noted previously (5).

The different constructs were incubated with nuclear extracts prepared from 369 and 422 cells in the presence of nucleotides. RNA was isolated and the specific products were evaluated by using primer extension. For this purpose, two oligonucleotides specific for the CAT and β-actin-*lacZ* transcripts were used. Transcription was stimulated 5 to 10 times more with the 369 nuclear extract than with the 422 nuclear extract (Fig. 3B). With the enhancerless template, only a weak transcriptional activation was observed in both extracts. The β-actin-*lacZ* control template showed equal amounts of RNA transcribed in vitro in all cases. The lengths of the RNAs produced were as expected, indicating that transcription was correctly initiated from the SV40 and β-actin promoters.

The amount of a transcription factor interacting with the *H-2K^k* enhancer is greatly reduced in the tumorigenic cell line 422. The results presented above demonstrate that the enhancer of the *H-2K^k* gene is inactive in the nonexpressing tumorigenic cell line 422. Many proteins interact specifically with the enhancer and promoter regions of eucaryotic genes and thus regulate transcription (20). The binding of *trans*-acting factors to the *H-2K^k* regulatory region in vitro was examined in both cell lines by DNase I footprinting (Fig. 4A). The pattern of protection of the *H-2K^k* regulatory region was different in the two cell lines. Specifically, an area centered around position −166 was clearly protected in 369 cells but not in 422 cells. The protection was prominent even with 20 μg of 369 nuclear extract but not with 80 μg of 422 extract (not shown). The protected area coincided with the binding site of the transcription factors H2TF1 and KBF1 (7,

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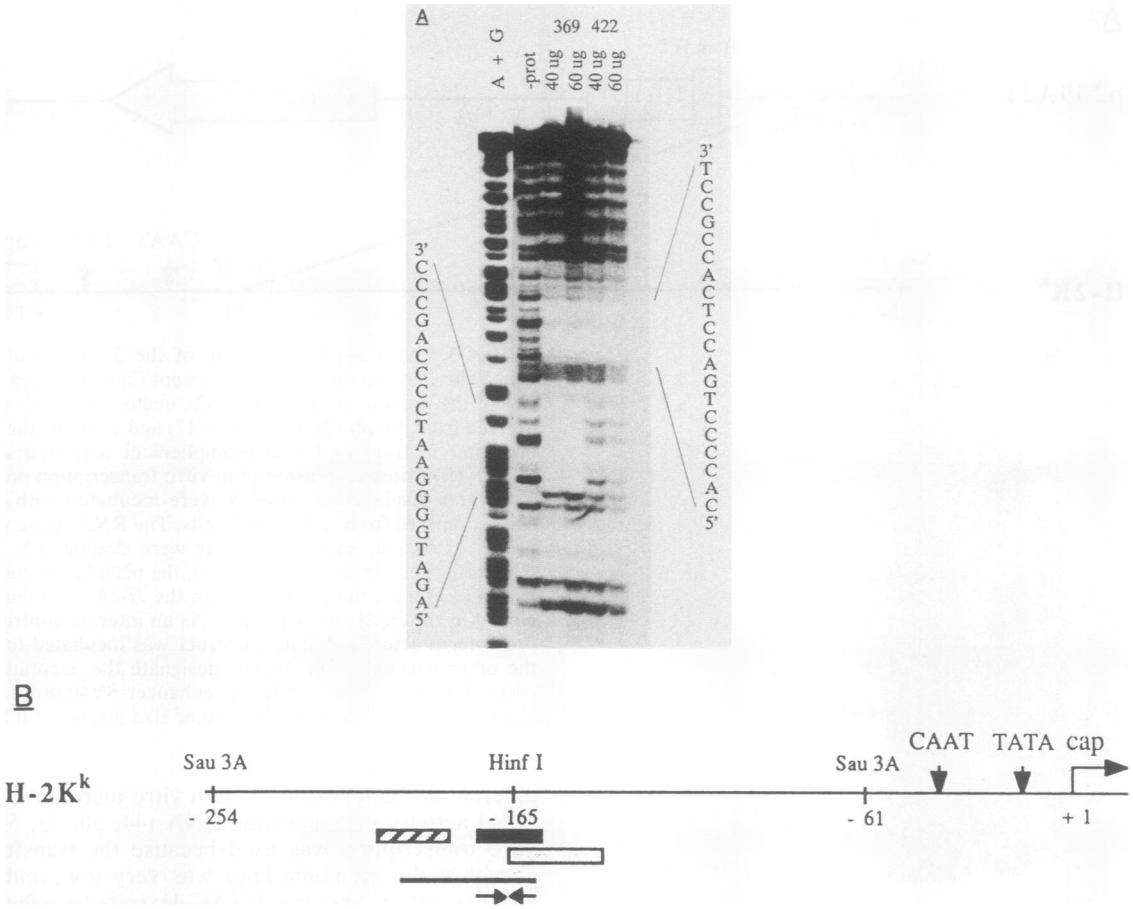

FIG. 4. DNase I footprinting analysis of the *H-2K^k* enhancer. (A) The 193-base-pair *Sau*3A fragment from the *H-2K^k* enhancer was radioactively marked at the noncoding strand, and DNase I footprinting reactions were carried out as described in Materials and Methods. Approximately 3 ng of end-labeled DNA was incubated with 40 or 60 μg of whole nuclear extract from 369 and 422 cells. The bracket on the left marks the protection seen only in 369 cells. The bracket on the right indicates the binding of another factor present in both cell types. Lane A+G, Sequencing reaction. (B) Schematic representation of the footprinting results. The 5′ region of the gene is shown. The black box represents the footprint found only in 369 cells, while the striped box represents the area protected in both 369 and 422 cells. The open box indicates the location of the interferon response sequence. The line marks the H1 oligonucleotide used in the gel retardation experiments (see text and Fig. 5). The arrows below depict the inverted repeats of the binding site of transcription factor H2TF1/KBF1.

24). These proteins were purified in different laboratories, and it is not clear at present whether they are identical. Just upstream, another area showing homology to the SV40 core motif was equally protected in both cell lines and served as an internal control (Fig. 4A). The protected areas are shown schematically in the context of the *H-2K^k* regulatory region (Fig. 4B).

The results of the DNase I footprinting analysis were confirmed by using the gel retardation assay. An oligonucleotide (H1 in Fig. 4B; see also Materials and Methods) spanning the symmetrical H2TF1/KBF1 binding site was radioactivelly labeled and incubated with the nuclear extracts, and the products were analyzed by gel electrophoresis. The results in Fig. 5 show that there was a strong retarded complex in 369 extracts and only a very weak one in 422.

Two additional oligonucleotides that contain the μE1 and μE2 motifs of the immunoglobulin heavy-chain enhancer were used. These motifs interact with the ubiquitous NF-μE1 and NF-μE2 proteins, respectively (3, 45, 59). Equal amounts of these factors were present in the 369 and 422 nuclear extracts, demonstrating that the observed difference was not an artifact of the extract preparation.

As mentioned above, the *cis*-acting element at position −166 of *H-2K^k* was recognized by the H2TF1/KBF1 transcription factor(s) (7, 24). This motif is similar to the binding site of the transcription factor NF-κB (30). However, the κB site is asymetric, containing only one of the two repeats. While H2TF1 recognizes the symmetrical *H-2K^b* site with high affinity and the κB site with low affinity, NF-κB recognizes both sites equally well (7). To test whether NF-κB is similarly affected, a second oligonucleotide containing the κB site of the SV40 enhancer was used in the gel retardation assay (S10; see also Materials and Methods). The results showed that S10 formed a complex that was weaker and ran slightly slower than the complex obtained with H1 (Fig. 5). Moreover, the intensity of the shifted band was considerably reduced in the negative cell line 422.

Because the H1 and S10 oligonucleotides gave mainly one shifted band in the gel retardation assay, each oligonucleotide might be recognized only by one distinct nuclear protein. To further analyze this possibility, the binding to the two probes was tested by competition experiments. H1 and S10 were radioactively labeled and incubated with 369 nuclear extracts. Competition was performed with unlabeled H1 or S10. Figure 6 shows that homologous competition was

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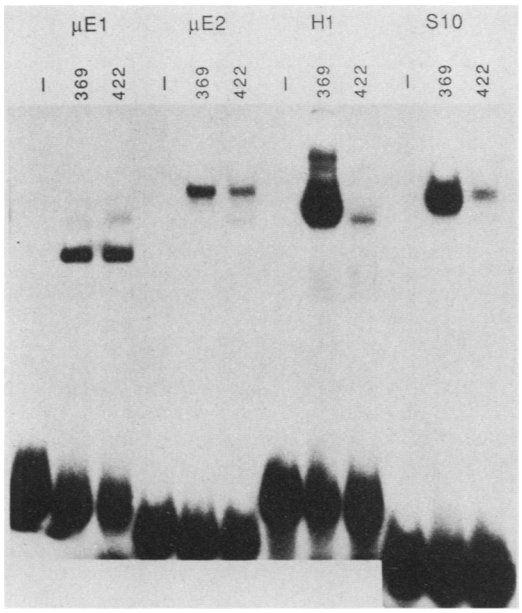

FIG. 5. Gel retardation with nuclear extracts from 369 and 422 cells. Four different oligonucleotides, μE1, μE2, H1, and S10 (sequences are shown in Materials and Methods), were radioactively end-labeled and incubated with nuclear extracts from 369 and 422 cells or without nuclear extract (—lanes). The products were analyzed in native polyacrylamide gels. Oligonucleotides μE1 and μE2 contain motifs of the immunoglobulin heavy-chain enhancer previously shown to bind ubiquitous factors. S10 contains the NF-κB site of the SV40 enhancer. The location of oligonucleotide H1 within the *H-2K*^{k} 5′ region is shown in Fig. 4B.

much more effective than competition by the heterologous oligonucleotide. In the case of homologous competition by H1, complex formation was reduced at least 20-fold more than by S10.

The results described above demonstrate that H1 and S10 recognize two different proteins and that both of these proteins are reduced in the tumorigenic cell line 422. Moreover, the binding to H1, which contains the H2TF1/KBF1 site, was not competed with efficiently by S10, which contains the NF-κB site. It is possible that H1 is recognized by H2TF1/KBF1 and S10 by NF-kB. If this is the case, then the results here are in apparent conflict with the previous report showing that NF-κB binds equally well to both sites (7). A possible explanation might be that the proteins identified in the AKR cells are distinct from H2TF1/KBF1 and NF-κB. Alternatively, the discrepancy might be a result of the different probes used in the gel retardation and competition experiments. In the previous report, fragments of the *H-2K*^{b} and the immunoglobulin κ light-chain enhancers were used (7), while we employed short synthetic oligonucleotides containing the corresponding sites of the *H-2K*^{k} and SV40 enhancers.

The amounts of the protein interacting with the *H-2K*^{k} enhancer follow the pattern of MHC class I expression in AKR leukemias and mouse tissues. The experiments described above suggest that at least one mechanism by which AKR leukemias suppress expression of class I antigens involves the inactivation of a transcription factor(s) which interacts with the H2TF1/KBF1 binding site. As mentioned above, many different thymomas exist that display variable amounts of H-2K^k antigen on their surface (48). In order to see how general is the phenomenon observed in the 369 and 422 cell lines, the gel retardation experiment was repeated with nuclear extracts from thymomas K36, 424, and 439 (Fig. 7A). K36 and 424 have small amounts of H-2K^k antigen on their surface and very low *H-2K*^{k} RNA levels, while 439 has intermediate levels (48). The results in Fig. 7A show that the levels of the DNA-protein complex formed with the H1 oligonucleotide correlated with the amounts of *H-2K*^{k} RNA in these cell lines. The oligonucleotide containing the μE2 site of the immunoglobulin heavy-chain enhancer was used as a control and showed again that the differences observed were specific for the H1 oligonucleotide (Fig. 7B). A similar result was obtained with the μE1 probe (not shown).

The levels of class I antigens also differ significantly among tissues. For example, large amounts are expressed in spleen, relatively low levels in liver, and no class I antigens have been detected in brain (25). It was interesting to see whether this mRNA distribution correlates with the level of the corresponding factor in these tissues the same way as it does in the different AKR thymomas.

Nuclear extracts were prepared from mouse brain, liver, and spleen and examined by the gel retardation method (Fig. 8). The intensity of the retarded complex in these tissues correlated exactly with the levels of class I expression. As a control, these tissues where also analyzed for the presence of the μE1, μE2, and octamer-binding proteins to show that the result was not an artifact of the nuclear extract preparation (not shown) (51). These findings suggest that inactivation or absence of H2TF1/KBF1 or a related factor(s) is a general mechanism that regulates transcription of certain class I and related genes. In brain and in liver, a second diffuse band was present that ran faster than the main complex. At this point, it is not clear if this band is due to a new protein or a modification of H2TF1/KBF1 (or NF-κB) in these tissues.

DISCUSSION

Thymoma cell lines derived from tumors in ageing AKR mice have different abilities to form leukemias when reintroduced back into animals (14, 47, 49). Detailed analysis showed that tumorigenicity correlates with expression of MHC class I genes in the cell membrane (47). AKR leukemias lose their tumorigenicity after H-2 antigen expression is restored (22). In other systems, similar observations were made. For instance, the metastatic properties of murine fibrosarcomas follow the pattern of H-2 expression, and reexpression by gene transfer of *H-2* genes abolishes metastasis (58).

Several lines of evidence indicate that the molecular mechanism underlying *H-2K*^{k} gene suppression is at the transcriptional level. RNA levels correlate with the amounts of H-2K^k protein on the cell surface. Several other class I genes and β_2m, which have transcriptional regulatory elements similar to those of *H-2K*^{k}, are also suppressed in H-2K^k-negative cell lines. The results presented in this report show that the *H-2K*^{k}, major enhancer is not functioning in the negative 422 cells, while it is active in the expressing 369 cells. The proteins interacting with this area of the *H-2K*^{k} gene were analyzed. The only detectable difference in the part of the 5′ region examined is that the nonproducing cells contain significantly lower amounts of a nuclear protein interacting with the H2TF1/KBF1 binding site. Since this sequence is essential for expression of class I genes, we conclude that suppression of class I genes in the tumorigenic cells is primarily due to the inactivation of transcription factor H2TF1/KBF1 or a related factor. It is

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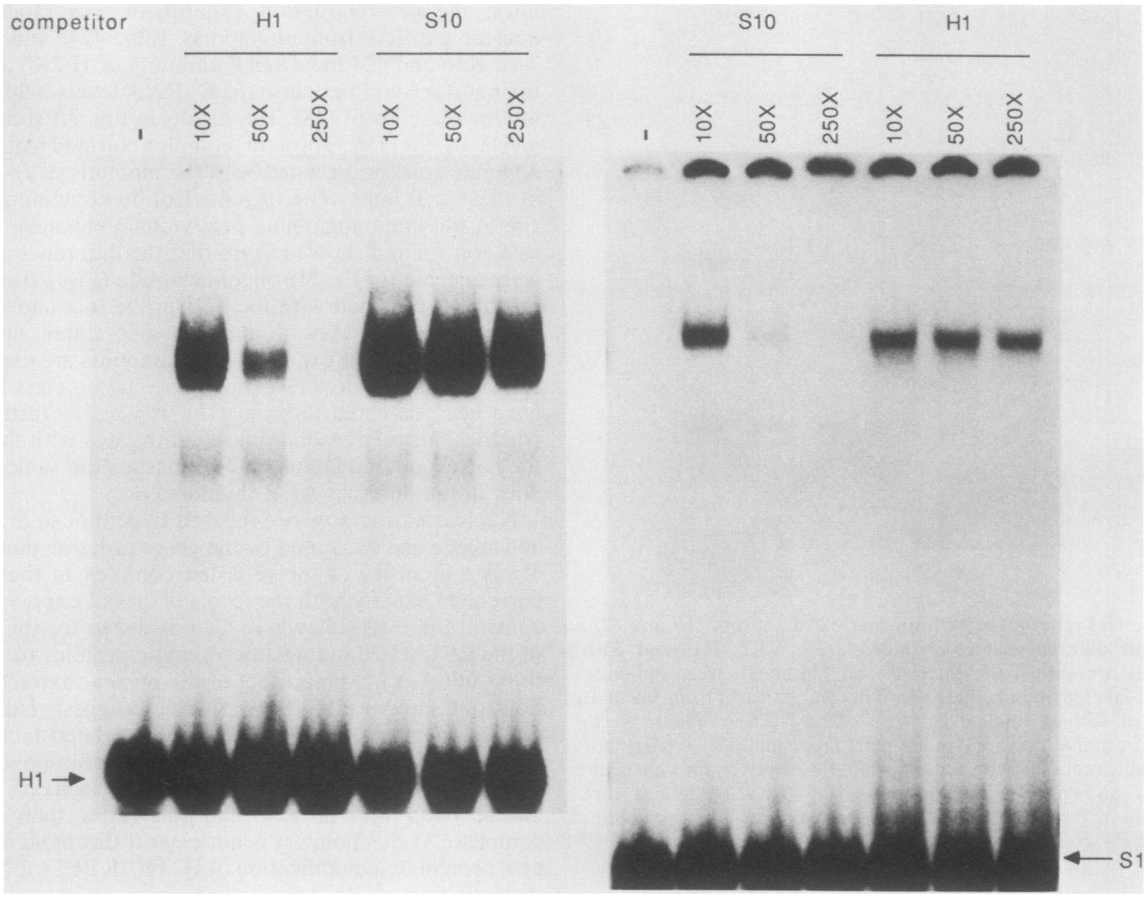

FIG. 6. Competition between H1 and S10 for binding of nuclear proteins in the gel retardation assay. Oligonucleotides H1 (left panel) and S10 (right panel) were radioactively end labeled and incubated with nuclear extracts from 369 cells in the presence of the indicated molar excess of competitor DNA. For this purpose, nonradioactive double-stranded oligonucleotides H1 and S10 were used. The competitor and its molar excess used in each lane are indicated on top. In (—) lanes the oligonucleotides were incubated without nuclear extract and competitor DNA. The free probes H1 and S10 are marked by arrows.

interesting that the levels of this nuclear protein in mouse tissues correlate well with the amounts of class I antigens reported to be present in these tissues (25). These results indicate that this factor, most probably H2TF1/KBF1, is one of the main regulators that control expression of H-2 antigens and related genes and thus support the same conclusion that was derived from transfection experiments (6, 26).

A probe (S10) which contains the NF-κB binding site showed a pattern in the gel retardation assay similar to that of the one (H1) which contains the H2TF1/KBF1 binding site. Since the different mobilities of the respective complexes and the competition experiments show that the two oligonucleotides are recognized by different proteins, it appears that the binding activity of both proteins is affected in the tumorigenic cell lines. Although H2TF1/KBF1 and NF-κB bind to similar sequences, they are distinct factors (30). It has been proposed that H2TF1/KBF1 might be responsible for constitutive binding and transcriptional activation, whereas NF-κB might act as an inducible activator (31). Although H2TF1/KBF1 is considered the main factor for expression of MHC class I genes, it cannot be excluded that NF-κB is also involved in their expression. Suppression of both proteins might then be responsible for the downregulation of the *H-2K*^{k} gene in the tumorigenic cell lines. The fact that only one band was detected with oligonucleotide H1 and this was not competed away efficiently by the NF-κB site of S10 indicates that NF-κB might not interact with the *H-2K*^{k} enhancer, and therefore its role remains unclear. In addition, it cannot be completely ruled out that the two proteins found in nuclear extracts of the AKR cell lines are distinct from both H2TF1/KBF1 and NF-κB.

Recently, it was shown that the different levels of MHC class I genes in certain tumor cell lines correlate with the presence of DNase I-hypersensitive sites around the genes (34). One of these sites coincides with the H2TF1/KBF1 binding site. Since DNase I-hypersensitive sites reflect the interaction of regulatory proteins with DNA, it would be interesting to examine the levels of H2TF1/KBF1 in these cell lines. It is also noteworthy that in severe combined immunodeficiency patients, the deficiency in MHC class II expression correlates with the absence of the promoter-binding protein RF-X, providing a similar example of suppression of gene expression due to inactivation of a transcription factor (41).

Nevertheless, additional secondary mechanisms may act to suppress class I gene expression. Cell fusion experiments between H-2-negative thymomas and a thymoma line of *H-2*^{k} haplotype led to cell hybrids which lost expression of the *H-2K*^{k} gene in 8 out of 10 cases by a postulated *trans*-acting mechanism (5). In the two cases which retained the *K*^{k} phenotype, the class I genes of the parental H-2-negative line were not reexpressed. It thus appears that in this case

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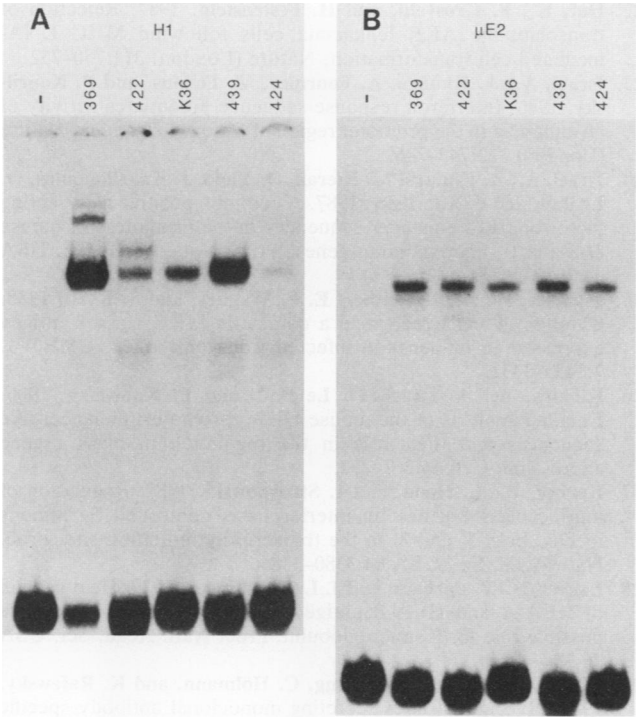

FIG. 7. Gel retardation of oligonucleotides H1 and μE2 with nuclear extracts of AKR leukemias. Radioactively end-labeled oligonucleotides H1 (A) and μE2 (B) were incubated with nuclear extracts of AKR leukemias 369, 422, K36, 439, and 424 as indicated above each lane. In (—) lanes the oligonucleotides were incubated without nuclear extract. The products were analyzed in native polyacrylamide gels.

additional *cis*-acting suppression mechanisms, for example, DNA methylation and packaging into heterochromatin, are involved (5).

The activity of H2TF1/KBF1 (or of a related factor) could be inhibited by several possible mechanisms in the tumorigenic cell lines. A simple explanation could be repression of the transcription factor's own gene. Recently, a cDNA clone of a protein interacting with the H2TF1/KBF1 binding site was isolated by a novel technique of screening cDNA expression libraries with the DNA-binding site as a probe (55). The cloning and the characterization of the H2TF1/KBF1 gene will certainly help to answer these questions. Second, a repressor might interact with the enhancer-promoter region of the *H-2K*^{k} gene. An active repression mechanism was proposed to explain the lack of *H-2L*^{d} expression in the embryonal carcinoma F9 cells (36). Interestingly, the repression site was mapped around the H2TF1/KBF1 binding site. There was no evidence, however, for repressor binding in the in vitro experiments described here. Third, the mechanism reported for the related transcription factor NF-κB might also operate in this case as well. Recently it was demonstrated that NF-κB is specifically and reversibly inhibited by a protein called IκB (4). The NF-κB-IkB complex is found in the cytoplasm and dissociates after phorbol ester stimulation. Subsequently, NF-κB translocates to the nucleus and activates transcription. However, it was shown that the DNA-binding activity of H2TF1/KBF1 was not affected by the NF-κB-specific inhibitor IκB (4). Thus, the regulation of H2TF1/KBF1 remains unclear, and the presence of another specific cytoplasmic inhibitor cannot be ruled out.

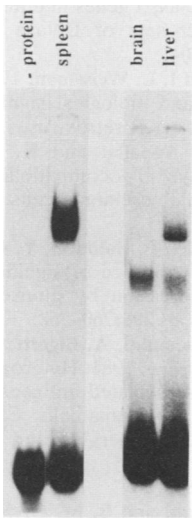

FIG. 8. Gel retardation of oligonucleotide H1 with nuclear extracts prepared from various mouse tissues. The source of the nuclear extract is indicated above the lanes. In the (—) protein lane, incubation was without nuclear extract.

The results presented here can also explain why interferon fails to stimulate expression of the H-2K^k antigen in the negative 422 cells. Interferon stimulation is mediated through an interferon response *cis*-acting sequence (IRS) (15) which partially overlaps the H2TF1/KBF1 binding site (23, 27, 36). Although the interferon response sequence is necessary for stimulation, it is active only when linked to a functional enhancer (23). If the class I enhancer is inactive in 422 cells due to the absence of H2TF1/KBF1 and NF-κB or of some related proteins, then as a consequence interferon treatment cannot stimulate expression of the class I antigens. It is true though that there is only a slight stimulation in the positive 369 cells as well. This may be because in these cells maximum expression has been achieved and no further stimulation is possible. This explanation is supported by the fact that class I expression can be stimulated in AKR leukemias with normal levels of class I antigens (48).

Recent studies indicate that the malignancy of certain human tumors correlates with human leukocyte antigen class I expression (38). Future work will be directed towards establishing how general is the phenomenon observed in the AKR leukemias in other tumorigenic cells. Experiments will also focus on ways to activate the transcription factor(s) in these cells. It would be of great therapeutic value if agents were identified that specifically induce the activity of these proteins in malignant cells.

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LITERATURE CITED

1. **Arce-Gomez, B., E. A. Jones, D. J. Barnstable, E. Solomon, and W. F. Bodmer.** 1977. The genetic control of HLA-A and -B antigens in somatic cells hybrids: requirement for β_2-microglobulin. Tissue Antigens **11**:92–112.
2. **Arnold, B., H. G. Burgert, A. L. Archibald, and S. Kvist.** 1984. Complete nucleotide sequence of the murine H-2K^k gene. Comparison of three H-2K locus alleles. Nucleic Acids Res. **12**:9473–9487.
3. **Augereau, P., and P. Chambon.** 1986. The mouse immunoglobulin heavy-chain enhancer: effect on transcription *in vitro* and binding of proteins present in HeLa and lymphoid B cell extracts. EMBO J. **5**:1791–1797.
4. **Baeuerle, P. A., and D. Baltimore.** 1988. IkB: a specific inhibitor of the NF-κB transcription factor. Science **242**:540–546.
5. **Baldacci, P., C. Transy, M. Cochet, C. Penit, A. Israel, and P. Kourilsky.** 1986. A *trans*-acting mechanism represses the expression of the major transplantation antigens in mouse hybrid thymoma cell lines. J. Exp. Med. **164**:677–694.
6. **Baldwin, A. S., and P. A. Sharp.** 1987. Binding of a nuclear factor to a regulatory sequence in the promoter of the mouse *H-2K^b* class I major histocompatibility gene. Mol. Cell. Biol. **7**:305–313.
7. **Baldwin, A. S., and P. A. Sharp.** 1988. Two transcription factors, NF-kB and H2TF1, interact with a single regulatory sequence in the class I major histocompatibility complex promoter. Proc. Natl. Acad. Sci. USA **85**:723–727.
8. **Bernards, R., P. I. Schrier, A. Houweling, J. L. Bos, A. J. Van der Erb, M. Zcylstra, and C. J. M. Melief.** 1983. Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature (London) **305**:776–779.
9. **Coligan, J. E., T. J. Kindt, H. Uehara, J. Martinko, and S. G. Nathensen.** 1981. Primary structure of a murine transplantation antigen. Nature (London) **291**:35–39.
10. **Daar, A. S., S. V. Fuggle, J. W. Fabre, A. Ting, and P. J. Morris.** 1984. The detailed distribution of HLA-A,B,C antigens in normal human organs. Transplantation **38**:287–292.
11. **De Baetselier, P., S. Katzav, E. Gorelik, M. Feldman, and S. Segal.** 1980. Differential expression of H-2 gene products in tumour cells is associated with their metastatic properties. Nature (London) **288**:179–181.
12. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
13. **Ephrussi, A., G. M. Church, S. Tonegawa, and W. Gilbert.** 1985. B lineage-specific interactions of an immunoglobulin enhancer with cellular factors in vivo. Science **227**:134–140.
14. **Festenstein, H., and W. Schmidt.** 1981. Variation in MHC antigenic profiles of tumor cells and its biological consequences. Immunol. Rev. **60**:85–127.
15. **Friedman, R. L., and G. R. Stark.** 1985. Alpha interferon induces transcription of HLA and metallothionein genes which have homologous upstream sequences. Nature (London) **314**: 637–639.
16. **Goodenow, R. S., J. M. Vogel, and R. L. Linsk.** 1985. Histocompatibility antigens on murine tumors. Science **230**:777–783.
17. **Gorman, C. M., P. W. J. Rigby, and D. P. Lane.** 1985. Negative regulation of viral enhancers in undifferentiated embryonic stem cells. Cell **42**:519–526.
18. **Guillemot, F., C. Auffray, H. T. Orr, and J. L. Strominger.** 1988. MHC antigen genes, p. 81–143. *In* B. D. Hames and D. M. Glover (ed.), Molecular immunology. IRL Press, Oxford.
19. **Hämmerling, G. J., D. Klar, W. Pülm, F. Momburg, and G. Moldenhauer.** 1987. The influence of major histocompatibility complex class I antigens on tumor growth and metastasis. Biochim. Biophys. Acta **907**:245–259.
20. **Hatzopoulos, A. K., U. Schlokat, and P. Gruss.** 1988. Enhancers and other regulatory elements, p. 43–96. *In* B. D. Hames and D. M. Glover (ed.), Transcription and splicing. IRL Press, Oxford.
21. **Hood, L., M. Steinmetz, and B. Mallisen.** 1983. Genes of the MHC of the mouse. Annu. Rev. Immunol. **1**:529–568.
22. **Hui, K., F. Grosveld, and H. Festenstein.** 1984. Rejection of transplantable AKR leukaemia cells following MHC DNA-mediated cell transformation. Nature (London) **311**:750–752.
23. **Israel, A., A. Kimura, A. Fournier, M. Fellous, and P. Kourilsky.** 1986. Interferon response sequence potentiates activity of an enhancer in the promoter region of a mouse *H-2* gene. Nature (London) **322**:743–746.
24. **Israel, A., A. Kimura, M. Kieran, O. Yano, J. Kanellopoulos, O. Le Bail, and P. Kourilsky.** 1987. A common positive *trans*-acting factor binds to enhancer sequences in the promoters of mouse *H-2* and β_2-microglobulin genes. Proc. Natl. Acad. Sci. USA **84**:2653–2657.
25. **Jefferies, W. A., U. Rüther, E. F. Wagner, and S. Kvist.** 1988. Cytolytic T cells recognize a chimaeric MHC class I antigen expressed in influenza A-infected transgenic mice. EMBO J. **7**:3423–3431.
26. **Kimura, A., A. Israel, O. Le Bail, and P. Kourilsky.** 1986. Detailed analysis of the mouse H-2K^b promoter: enhancer-like sequences and their role in the regulation of class I gene expression. Cell **44**:261–272.
27. **Korber, B., L. Hood, and I. Stroynowski.** 1987. Regulation of murine class I genes by interferons is controlled by regions located both 5′ and 3′ to the transcription initiation site. Proc. Natl. Acad. Sci. USA **84**:3380–3384.
28. **Lancet, D., P. Parham, and J. L. Strominger.** 1979. Heavy chain of HLA-A and HLA-B antigens is conformationally label: a possible role for β_2-microglobulin. Proc. Natl. Acad. Sci. USA **76**:3844–3848.
29. **Lemke, H., G. J. Hämmerling, C. Hölmann, and K. Rajewsky.** 1979. Hybrid cell lines secreting monoclonal antibody specific for major histocompatibility antigens of the mouse. Nature (London) **271**:249–251.
30. **Lenardo, M. J., and D. Baltimore.** 1989. NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell **58**:227–229.
31. **Lenardo, M. J., C.-M. Fan, T. Maniatis, and D. Baltimore.** 1989. The involvement of NF-κB in β-interferon gene regulation reveals its role as widely inducible mediator of signal transduction. Cell **57**:287–294.
32. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Manley, J. L., A. Fire, A. Cano, P. A. Sharp, and M. L. Gefter.** 1980. DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract. Proc. Natl. Acad. Sci. USA **77**: 3855–3859.
34. **Maschek, U., W. Pülm, and G. J. Hämmerling.** 1989. Altered regulation of MHC class I genes in different tumour cell lines is reflected by distinct sets of DNase I hypersensitive sites. EMBO J. **8**:2297–2304.
35. **McGrath, M. S., and I. L. Weissman.** 1979. AKR leukaemogenesis. Identification and biological significance of thymic lymphoma receptors for AKR retroviruses. Cell **17**:65–75.
36. **Miyazaki, J.-I., E. Appella, and K. Ozato.** 1986. Negative regulation of the major histocompatibility class I gene in undifferentiated embryonal carcinoma cells. Proc. Natl. Acad. Sci. USA **83**:9537–9541.
37. **Morello, D., F. Daniel, P. Baldacci, Y. Cayre, G. Gachelin, and P. Kourilsky.** 1982. Absence of significant H-2 and β_2-microglobulin mRNA expression by mouse embryonal carcinoma cells. Nature (London) **296**:260–262.
38. **Natali, P. G., P. Giacomini, A. Bigotti, K. Imai, M. R. Nicotra, A. K. Ng, and S. Ferrone.** 1983. Heterogeneity in the expression of HLA and tumor-associated antigens by surgically removed and cultured breast carcinoma cells. Cancer Res. **43**:660–668.
39. **Owen, M. J., A. M. Kissonerghis, and H. F. Lodish.** 1980. The biosynthesis of HLA-A, -B, and -C antigens in vivo. J. Biol. Chem. **235**:9678–9684.
40. **Ozato, K., Y.-J. Wan, and B. M. Orrison.** 1985. Mouse major histocompatibility class I gene expression begins at mid-somite stage and is inducible in earlier stage embryos by interferon. Proc. Natl. Acad. Sci. USA **82**:2427–2431.
41. **Reith, W., S. Satola, C. H. Sanchez, I. Amaldi, B. Lisowska-**

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Grospierre, C. Griscelli, M. R. Hadam, and B. Mach. 1988. Congenital immunodeficiency with a regulatory defect in MHC class II gene expression lacks a specific HLA-DR promoter binding protein, RF-X. Cell **53**:897–906.

42. **Rosa, F., D. Le Boutiller, A. Abadie, Z. Mishal, F. A. Lemonnier, D. Bourrel, M. Lamotte, J. Kalil, B. R. Jordan, and M. Fellous.** 1983. HLA class I genes integrated into murine cells are inducible by interferon. Eur. J. Immunol. **13**:495–499.
43. **Rosenthal, A., S. Wright, H. Cedar, R. Flavell, and F. Grosfeld.** 1984. Regulated expression of an introduced MHC H-2K^{bm1} gene in murine embryonal carcinoma cells. Nature (London) **310**:415–418.
44. **Sassone-Corsi, P., A. Wildeman, and P. Champon.** 1985. A *trans*-acting factor is responsible for the simian virus 40 enhancer activity *in vitro*. Nature (London) **313**:458–463.
45. **Schlokat, U., D. Bohmann, H. Schöler, and P. Gruss.** 1986. Nuclear factors binding specific sequences within the immunoglobulin enhancer interact differentially with other enhancer elements. EMBO J. **5**:3251–3258.
46. **Schmidt, W., G. Atfield, and H. Festenstein.** 1979. Loss of H-2K^k gene product(s) from an AKR spontaneous leukaemia. Immunogenetics **8**:311–321.
47. **Schmidt, W., and H. Festenstein.** 1982. Resistance to cell-mediated cytotoxicity is correlated with reduction of H-2K gene products in AKR leukaemia. Immunogenetics **16**:257–264.
48. **Schmidt, W., U. Henseling, D. Bevec, A. D. R. Alonzo, and H. Festenstein.** 1985. Control of synthesis and expression of H-2 heavy chain and beta-2 microglobulin in AKR leukaemias. Immunogenetics **22**:483–494.
49. **Schmidt, W., L. Leben, G. Atfield, and H. Festenstein.** 1981. Variation of expression of histocompatibility antigens on tumor cells: absence of H-2K^k gene products from a Gross virus-induced leukaemia in BALB.K. Immunogenetics **14**:323–339.
50. **Schöler, H., and P. Gruss.** 1985. Cell type-specific transcriptional enhancement *in vitro* requires the presence of *trans*-acting factors. EMBO J. **4**:3005–3013.
51. **Schöler, H. R., A. K. Hatzopoulos, R. Balling, N. Suzuki, and P. Gruss.** 1989. A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. EMBO J. **8**:2543–2550.
52. **Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. Van der Erb.** 1983. Expression of class I major histocompatibility antigens switched off by a highly oncogenic adenovirus 12 in transformed cells. Nature (London) **305**:771–775.
53. **Sen, R., and D. Baltimore.** 1987. In vitro transcription of immunoglobulin genes in a B-cell extract: effects of enhancer and promoter sequences. Mol. Cell. Biol. **7**:1989–1994.
54. **Sergeant, A., D. Bohmann, H. Zentgraf, H. Weiher, and W. Keller.** 1984. A transcription enhancer acts *in vitro* over distances of hundreds of base-pairs on both circular and linear templates but not on chromatin-reconstituted DNA. J. Mol. Biol. **180**:577–600.
55. **Singh, H., J. H. LeBowitz, A. S. Baldwin, Jr., and P. A. Sharp.** 1988. Molecular cloning of an enhancer binding protein: isolation by screening of an expression library with a recognition site DNA. Cell **52**:415–423.
56. **Steinmetz, M., J. H. Frelinger, D. Fisher, T. Hunkapiller, D. Pereira, S. M. Weissman, H. Uehara, S. Nathenson, and L. Hood.** 1981. Characterization of three cDNA clones encoding mouse transplantation antigens: homologies to immunoglobulins. Cell **24**:125–134.
57. **Travers, P. J., J. L. Arklie, J. Trowsdale, R. A. Patillo, and W. F. Bodmer.** 1982. Lack of expression of HLA-A, B, C antigens in choriocarcinoma and other human tumor cell lines. Natl. Cancer Inst. Monograph **60**:175–180.
58. **Wallich, R., N. Bulbuc, G. J. Hämmerling, S. Katzav, S. Segal, and M. Feldman.** 1985. Abrogation of metastatic properties of tumour cells by *de novo* expression of H-2K antigens following *H-2* gene transfection. Nature (London) **315**:301–305.
59. **Weinberger, J., D. Baltimore, and P. A. Sharp.** 1986. Distinct factors bind to apparently homologous sequences in the immunoglobulin heavy-chain enhancer. Nature (London) **322**:846–848.
60. **Wildeman, A., P. Sassone-Corsi, T. Grundström, M. Zenke, and P. Chambon.** 1984. Stimulation of *in vitro* transcription from the SV40 early promoter by the enhancer involves a specific *trans*-acting factor. EMBO J. **3**:3129–3133.
61. **Xin, J. H., S. Kvist, and B. Dobberstein.** 1982. Identification of an H-2K^d gene using a specific cDNA probe. EMBO J. **1**: 467–471.
62. **Yano, O., J. Kanellopoulos, M. Kieran, O. Le Bail, A. Israel, and P. Kourilsky.** 1987. Purification of KBF1, a common factor binding to both H-2 and β_2-microglobulin enhancers. EMBO J. **6**:3317–3324.
63. **Zinkernagel, R. M., and P. C. Doherty.** 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction specificity, function and responsiveness. Adv. Immunol. **27**: 51–177.

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