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Adenovirus E1A-mediated regulation of class I MHC expression

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Expression of class I MHC transplantation antigens has been shown to be reduced in baby rat kidney (BRK) cells transformed by highly oncogenic adenovirus type 12 (Ad12), as compared with untransformed cells and cells transformed by non-oncogenic Ad5. Here we show that this reduction of class I expression also occurs in a variety of other primary cell cultures transformed by Ad12, and that reduction of class I gene expression occurs for all class I loci. Transfection of Ad5E1 into class I-negative Ad12-transformed BRK cells leads to complete restoration of class I expression. Introduction of Ad12E1 into most class I-positive established cell lines does not result in suppression of class I expression. However, transfection of the Ad12E1A region into a class I-positive cell line which was immortalized by a mutant Ad12E1A region resulted in suppression of class I gene expression, implying that the suppression of class I activity in Ad12-transformed cells is due to an active switching-off process.

Key words: adenovirus E1A/class I MHC/gene expression

Introduction

Human adenoviruses differ in their potential to induce tumors in newborn hamsters, adenovirus type 5 (Ad5) being an example of a non-oncogenic virus and Ad12 of a highly oncogenic virus (Flint, 1980). Although both Ad5 and Ad12 are capable of transforming primary cultures of baby rat kidney (BRK) cells *in vitro*, only the Ad12-transformed BRK cells are oncogenic when injected into syngeneic animals.

The transforming region of both viruses is located in early region 1 (E1), the first region to be expressed during productive infection, comprising approximately the leftmost 10% of the viral genome (reviewed in Petterson and Akusjärvi, 1978). The E1 regions of both Ad5 and Ad12 consist of two transcriptional units, E1A and E1B, which show remarkable structural and functional homology (Bernards *et al.*, 1982; Bernards and Van der Eb, 1984).

In an attempt to define the relevant differences between Ad5- and Ad12-transformed BRK cells which might explain their different oncogenic potential in syngeneic animals, we have found that Ad12-transformed cells express drastically reduced amounts of the MHC-encoded class I transplantation antigens as compared with untransformed and Ad5-transformed BRK cells (Schrier *et al.*, 1983; Bernards *et al.*, 1983). Reduction of class I gene expression was found to occur at the mRNA level and to correlate with expression of the product of the 13S E1A mRNA of Ad12 (Schrier *et al.*, 1983).

Although we have proposed on the basis of our previously published data that the Ad12E1A product switches off the expression of class I genes, we could not exclude the possibility that Ad12 selectively transforms cells that have low class I expression. This would imply that Ad12 only transforms specific target cells which differ from those transformed by Ad5 with respect to class I expression. It has been reported that cells of neuronal origin are particularly effective targets for transformation by Ad12 (Gallimore and Paraskeva, 1980; Mukai, 1976). As neuronal cells are known to express low amounts of class I antigens (Vitetta and Capra, 1978; Williams *et al.*, 1980) this could explain the effects which we have previously reported. Therefore, experiments were undertaken to distinguish between an active switching-off of class I gene expression, and selective

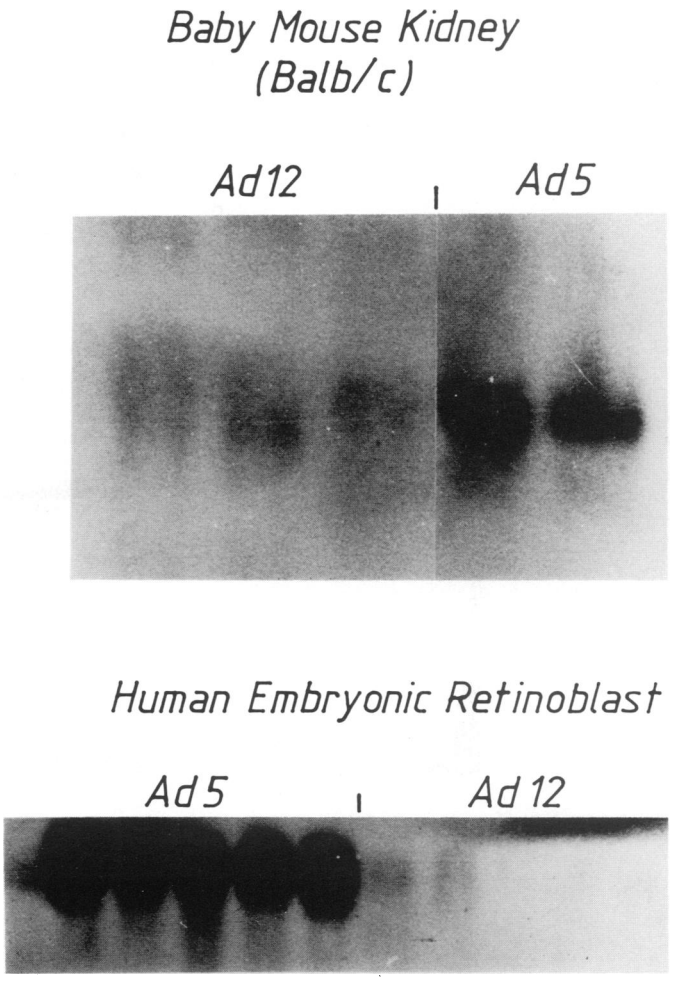

Fig. 1. Class I MHC transcripts in Ad5- and Ad12-transformed BMK and HER cells. 20 μg of cytoplasmic RNA of different cell lines were applied to single lanes and subjected to Northern blotting analysis. Filters were hybridized with a nick-translated human HLA-B7 cDNA probe (Sood *et al.*, 1981).

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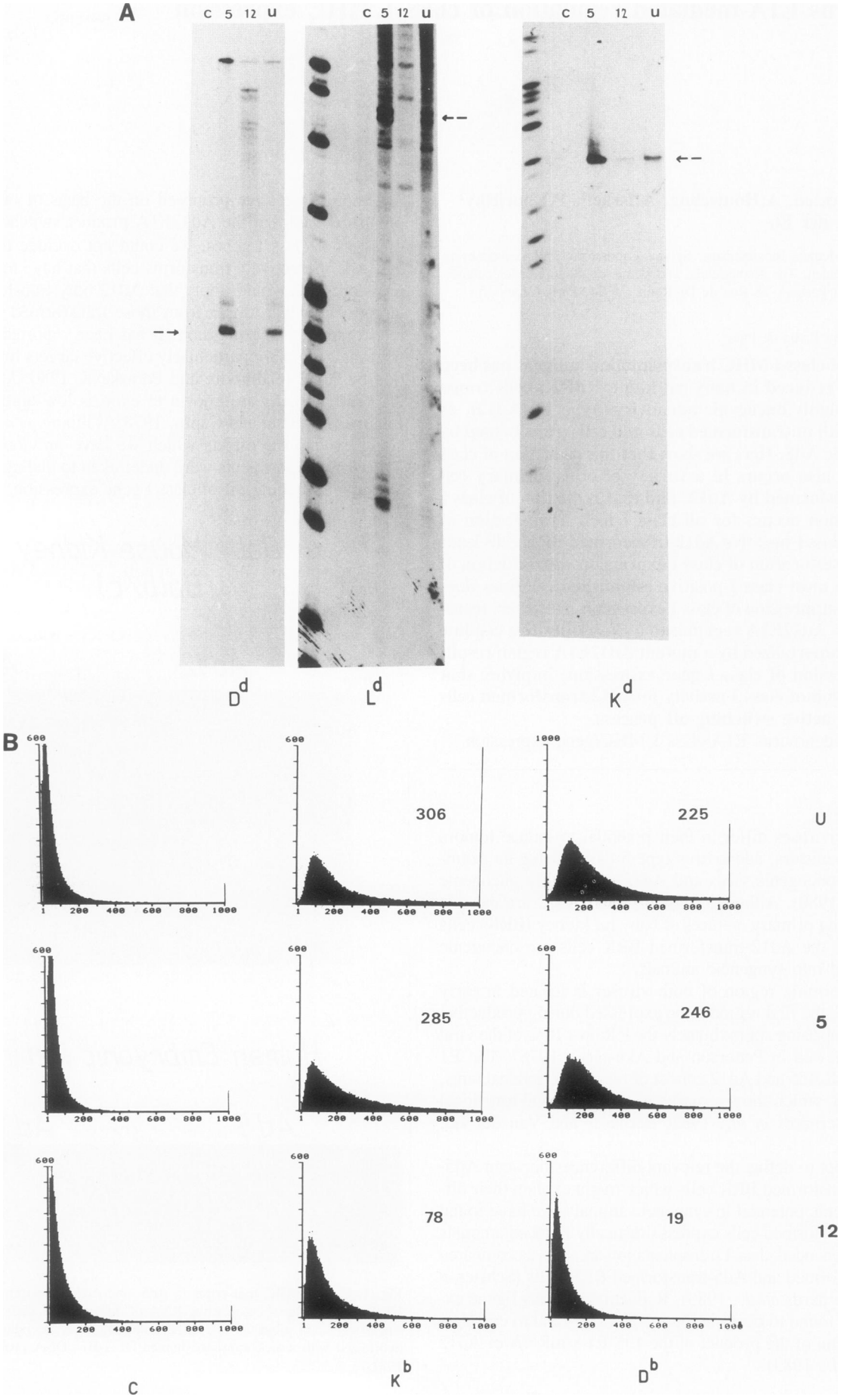
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D^b

transformation of specific (class I-'negative') target cells.

The results presented here show that reduction of class I expression happens in a variety of primary cell cultures transformed by Ad12E1, and that this reduction occurs for all class I loci. Transfection of class I-negative Ad12-transformed cells with Ad5 region E1 results in the complete restoration of class I expression, indicating that class I genes can be expressed in Ad12-transformed cells, and that products of Ad5E1A can compete out those of Ad12E1A in the modulation of class I expression. Introduction of Ad12E1 into several class I-positive established cell lines does not result in suppression of class I expression. However, by using a cell line recently transformed by an Ad12E1A deletion mutant which still showed normal class I gene expression, we were able to show that a normal Ad12E1 region is indeed capable of switching-off class I expression upon introduction into class I-positive cells. This indicates that reduced class I MHC expression in Ad12-transformed cells is indeed due to an active switching-off process, and not to the selective transformation of cells that happen to show low class I expression.

Results

Class I expression in various Ad12-transformed primary cell cultures

To determine whether reduction of class I expression in Ad12-transformed BRK cells, as compared with untransformed and Ad5-transformed cells, is a general phenomenon, we have transformed a variety of primary cultures with Ad5E1 and Ad12E1 and compared the levels of class I expression in the transformed cells. Primary cultures of baby mouse kidney (BMK), rat embryo brain (REB), hamster embryo and human embryonic retinoblast (HER) cells were used. In all cases the level of class I expression, as measured by Northern blotting analysis of cytoplasmic RNA and immunoprecipitation of [^{35}S]-methionine-labeled cell extracts, was significantly reduced in the Ad12-transformed cells as compared with their Ad5-transformed equivalents. Figure 1 shows the results of Northern blotting analyses with RNA from Ad5- and Ad12-transformed BMK and HER cells. Similar results were obtained for Ad5- and Ad12-transformed REB and hamster embryo cells (data not shown). This indicates that low expression of class I transplantation antigens is a common property of many primary cell cultures transformed by Ad12E1.

Immunoprecipitation of [^{35}S]methionine-labeled cell extracts of cells transformed by another highly oncogenic adenovirus, Ad31, has shown that class I expression in these cells is as low as in the Ad12-transformed cells (data not shown), indicating that low class I expression is a general property of cells transformed by highly oncogenic adenoviruses.

Expression of all class I loci is decreased in Ad12-transformed cells

Class I antigens are encoded by different loci in man (HLA-A,B,C), mouse (H-2K,D,L) and rat (RT1.A,E). Although the expression of class I antigens is strongly reduced in all Ad12-transformed cells tested thus far, a residual amount of class I products is always present in these cells. It is known that in tumor cells the expression of individual class I loci can be regulated independently, e.g. in the AKR leukemia cell line K36.16 the H-2K^k antigen cannot be detected, whereas expression of H-2D^k is normal (Festenstein and Schmidt, 1981). Hence, the residual class I expression in Ad12-transformed cells could be due to the fact that a particular class I locus is not suppressed in Ad12-transformed cells. It seemed of interest, therefore, to address the question of whether reduction of class I expression in Ad12-transformed cells occurs for all class I loci to the same extent, or is locus-specific. We used two approaches to answer this question, using untransformed, and Ad5- and Ad12-transformed BMK cells. Firstly, S1 nuclease analyses were performed with cytoplasmic RNA using locus-specific DNA probes (see legend to Figure 2). Figure 2A shows that the RNA expression of all three loci (K,D,L) is somewhat higher in the Ad5-transformed than in the untransformed cells. However, RNA expression of all loci is drastically reduced in the Ad12-transformed cells. Secondly, expression of class I protein products of the individual loci at the cell surface was investigated by fluorescence-activated cell sorting (FACS) with specific alloantisera. Figure 2B shows the results of these analyses obtained for untransformed, Ad5- and Ad12-transformed BMK cells from C57/BL mice of the H-2^b haplotype. The FACS results confirm those obtained by S1 analysis, i.e. expression of all loci (C57/BL mice do not have an L locus) is drastically reduced in the Ad12-transformed cells. The FACS results with BALB/c BMK cells were identical to those obtained with the C57/BL BMK cells (data not shown). The finding that suppression of class I expression occurs in Ad12-transformed mouse cells of different haplotypes confirms our earlier results obtained with rat cells (Schrier *et al.*, 1983).

Class I expression in Ad12-transformed cells can be activated

We have previously shown that the inhibition of class I gene expression in Ad12-transformed cells correlates with the presence of the 13S mRNA transcribed from the Ad12E1A region (Schrier *et al.*, 1983). This observation suggested that the inhibition of class I expression is due to a switching-off phenomenon mediated by the 13S mRNA product. However, the primary cultures used for the transformation assays presumably consist of a heterogeneous cell population, and it cannot be excluded that Ad12E1 transforms cell types other than Ad5E1. This possibility is supported by the observation that Ad12, as opposed to Ad5, seems to select cells of neuronal origin for transformation (Gallimore and Paraskeva, 1980; Mukai, 1976), and that these cells are known to express low amounts of class I antigens (Vitetta and Capra, 1978; Williams *et al.*, 1980). Furthermore, it could provide an explanation for the fact that Ad5E1 transforms BRK cells with a 10- to 50-fold higher efficiency than Ad12E1 (Bernards *et al.*, 1982; Bernards and van der Eb, 1984). Thus, we could not exclude the possibility that Ad12 recognizes a specific type

Fig. 2. Expression of individual H-2 class I loci in untransformed, and Ad5E1- and Ad12E1-transformed BMK cells. **(A)** S1 nuclease analysis of 20 μg cytoplasmic RNA extracted from **u**, untransformed; **5**, Ad5E1-transformed; **12**, Ad12E1-transformed BALB/c (H-2^d) BMK cells. Arrows indicate the positions of the S1 nuclease-resistant segments. **c**, S1 nuclease-resistant segments after hybridization of the probes to tRNA. The following DNA probes were used: K^d, an *AvaI-KpnI* fragment isolated from pB4, an expression vector derived from pH-2^d-33 (Lalanne *et al.*, 1983), 5′ end-labeled at the *AvaI* site. Hybridization at 60°C; protected segment 145 nucleotides (M.Cochet, unpublished results). D^d, a *PvuII-EcoRI* fragment isolated from pH-2^d-7, a shorter version of pH-2^d-1 (Lalanne *et al.*, 1982), 5′ end-labeled at the *PvuII* site. Hybridization at 52°C; protected segment 215 nucleotides. L^d, a *PvuII-EcoRI* fragment isolated from pH-2^d-3 (Lalanne *et al.*, 1982), 5′ end-labeled at the *PvuII* site. Hybridization at 56°C; protected segment 484 nucleotides. **(B)** FACS analysis of untransformed, Ad5E1- and Ad12E1-transformed C57/BL (H-2^b) BMK cells. Cells were incubated with specific anti-K^b or -D^b anti-serum and fluorescent conjugate, respectively, or conjugate only **(panel C)**. Numbers indicate the average fluorescence value for each sample which was calculated as follows: $m_{antibody}/m_{conjugate} - 1$.

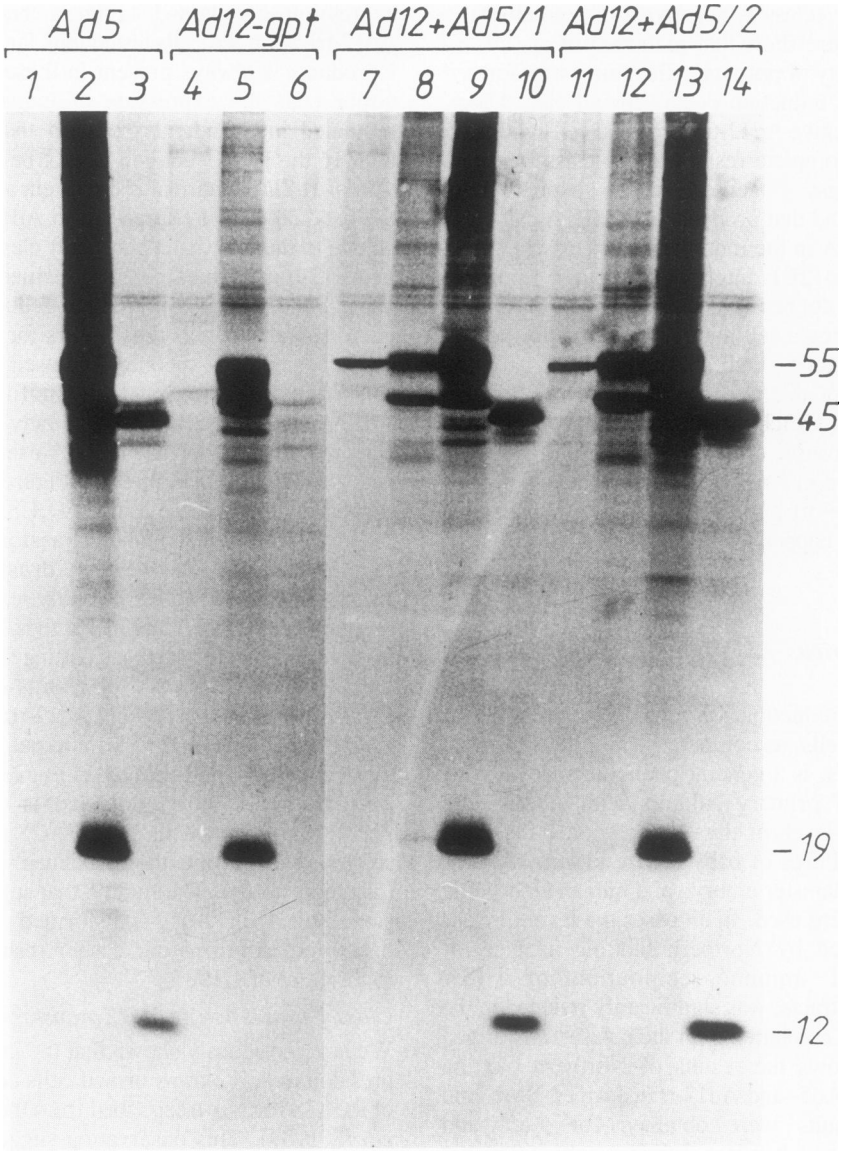

Fig. 3. SDS-polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of [^{35}S]methionine-labeled cells with normal rat serum **(lanes 1, 4, 7 and 11)**, Ad5 anti-T serum **(lanes 2, 9 and 13)**, Ad12 anti-T serum **(lanes 5, 8 and 12)** and a mouse monoclonal antibody against rat class I MHC heavy chains **(lanes 3, 6, 10 and 14)**. The following cell lines were used: Ad5, BRK cells transformed by Ad5E1; Ad12-gpt, Ad12E1-transformed BRK cells transfected with pSV2gpt (Mulligan and Berg, 1981); Ad12+Ad5, Ad12E1-transformed BRK cells transfected with Ad5E1 and pSV2gpt (1 and 2 represent different cell lines). The mol. wts (in kd) of the adenovirus small and large T antigens (19 and 55, respectively), the class I MHC heavy chain (45) and β_2-microglobulin (12) are indicated.

of target cell for transformation and that these cells are incapable of expressing normal amounts of class I antigens.

To distinguish between active switching-off and selective transformation, we made use of our earlier finding showing that cells obtained after transfection of BRK cells with plasmids containing Ad5E1A in addition to Ad12E1 express normal amounts of class I antigens (Schrier *et al.*, 1983). This result suggested that Ad5E1A products are dominant over Ad12E1A products with respect to the regulation of class I expression. To test this possibility more directly, we supertransfected class I-negative Ad12-transformed BRK cells with Ad5E1, in the presence of a dominant selection marker (pSV2gpt; Mulligan and Berg, 1981). Individual clones were screened for the expression of Ad5E1 and class I products. Figure 3 shows the result of immunoprecipitations of [^{35}S]methionine-labeled cell extracts of the original and the supertransfected Ad12-transformed cells. This experiment shows clearly that the level of class I expression in the Ad12-transformed cells supertransfected with Ad5E1 increases to the level found in Ad5-transformed cells (lanes 3, 10 and 14), indicating firstly that Ad5E1 can activate class I expression in Ad12-transformed cells, and secondly that Ad12-transformed cells are intrinsically capable of expressing class I genes.

Introduction of Ad12E1 into established cell lines

Although the results presented in the previous section demonstrate that Ad12-transformed BRK cells are capable of expressing class I genes, they do not prove that the low class I expression in these cells is actually caused by a switching-off process mediated by Ad12E1A. As primary cultures have the disadvantage of heterogeneity of the cell population, we transfected cultures of class I-positive established cell lines, which presumably are more homogeneous, with Ad12E1 to investigate whether reduction of

class I expression also occurs in these cells. Cultures of the rat cell lines 3Y1 (Kimura *et al.*, 1975) and BRLtk$^-$ and the mouse cell line Ltk$^-$ were transfected with Ad5E1 or Ad12E1 in the presence of a dominant selection marker. Individual cell lines were screened for the expression of class I mRNA by Northern blotting analysis of cytoplasmic RNA, using the human HLA-B7 cDNA clone (Sood *et al.*, 1981) as a probe. Figure 4 shows the result for a number of clones obtained after transfection of rat BRLtk$^-$ cells. It can be seen that there is no difference in class I expression between cells transfected with either Ad5E1 or Ad12E1. Similar results were obtained after transfection of rat 3Y1 and mouse Ltk$^-$ cells with Ad5E1 or Ad12E1 (data not shown). These results demonstrate that introduction of Ad12E1 into cell cultures that were already immortalized prior to transfection, as opposed to cultures of primary cells, does not result in reduction of class I expression.

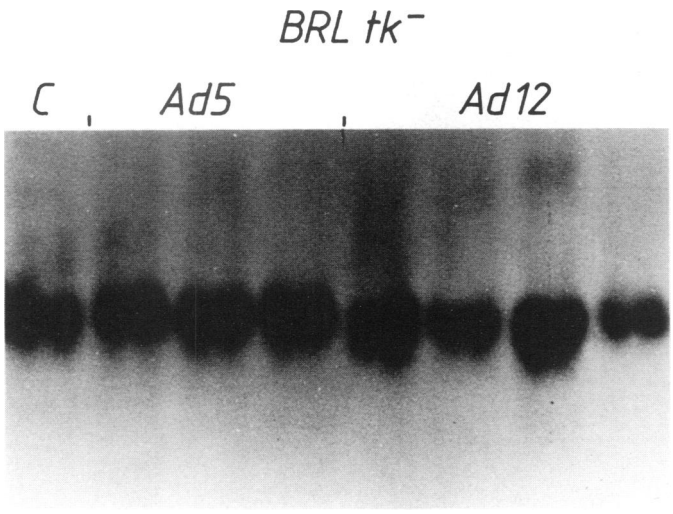

Fig. 4. Class I MHC transcripts in BRLtk$^-$ cells (C), and BRLtk$^-$ cells transfected with Ad5E1 or Ad12E1. Experimental conditions were as described in the legend to Figure 1.

To distinguish between active suppression of class I gene expression and selection of target cells expressing low amounts of class I products, we have made use of a BRK cell line which was recently transformed by a mutant Ad12E1 region, R11. This mutant E1 region carries a deletion in region E1A so that it can only direct the synthesis of a 15-kd N-terminal truncated protein (Bos *et al.*, 1983). The R11 mutant plasmid is defective in transformation, unless the SV40 early promoter plus enhancer region is inserted upstream of R11E1A (SVR11). The E1A region of SVR11 can stimulate expression of region E1B (Bos *et al.*, 1983), but the transformed cells do not show reduction of class I gene expression (Schrier *et al.*, 1983), and they are non-oncogenic, even in nude mice (Bos *et al.*, 1983). We have investigated the effect on class I expression of introduction of a wild-type Ad12E1 region into the SVR11-transformed cells. Co-transfection of Ad12E1 and pSV2neo (Southern and Berg,

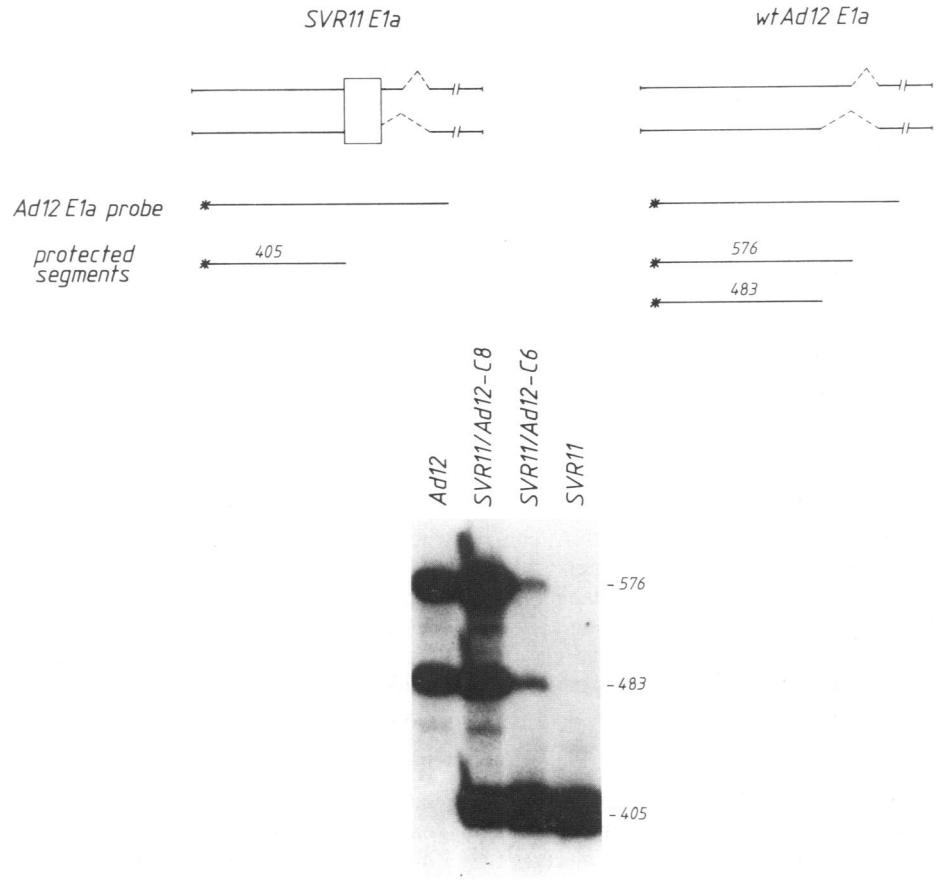

Fig. 5. Schematic representation of the S1 nuclease analysis performed to verify the expression of wild-type Ad12E1A mRNAs after transfection of Ad12E1 into SVR11-transformed BRK cells. The E1A mRNAs from SVR11 and wild-type Ad12 are shown. The box in SVR11 E1A represents a 109-bp deletion (Bos *et al.*, 1983). A *Nar*I-*Dde*I fragment isolated from pAd12Acc (Jochemsen *et al.*, 1984) 3′ end-labeled at the *Nar*I position was used as probe. The length of the protected segments is indicated. The autoradiograph shows the S1-resistant fragments run on a 5% acrylamide−7 M urea gel after hybridization of the DNA probe to 20 μg cytoplasmic RNA at 55°C.

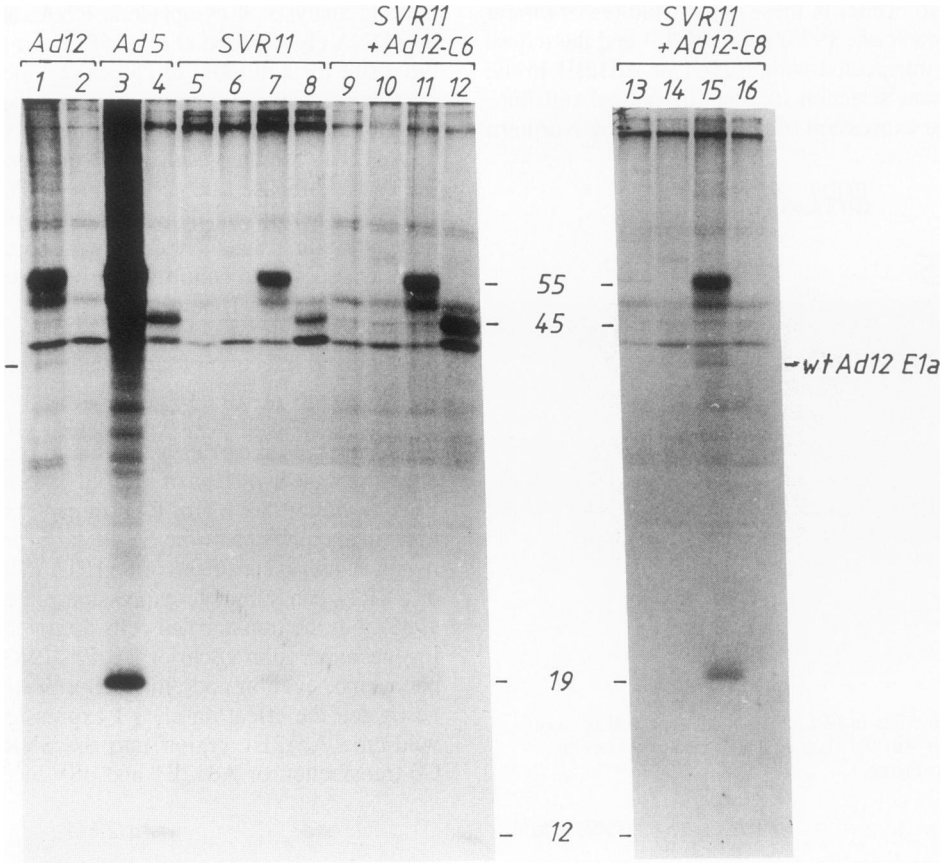

Fig. 6. SDS-polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of [^{35}S]methionine-labeled cells with normal rat serum **(lanes 5, 9 and 13)**, Ad5 anti-T serum **(lanes 3, 6, 10 and 14)**, Ad12 anti-T serum **(lanes 1, 7, 11 and 15)** and a Lew anti Wag rat alloantiserum (Schrier *et al.*, 1983) **(lanes 2, 4, 8, 12 and 16)**. The following cell lines were used: Ad5, BRK cells transformed by Ad5E1; Ad12, BRK cells transformed by Ad12E1; SVR11, BRK cells transformed by plasmid pSVR11 (Bos *et al.*, 1983); SVR11+Ad12, SVR11 cells transfected with Ad12E1 and pSV2neo (Southern and Berg, 1982); C6 and C8 referring to individual G418-resistant cell lines. wtAd12E1a refers to wild-type Ad12E1A protein precipitated in **lanes 1** and **15**. The mol. wts (in kd) of the adenovirus small and large T antigens (19 and 55, respectively), the class I MHC heavy chain (45) and β_2-microglobulin (12) are indicated.

1982) resulted in the isolation of two cell lines in which both the mutant and the wild-type Ad12E1A region were expressed, as demonstrated by S1 nuclease analysis. Figure 5 shows that the amount of RNA transcribed from the wild-type E1A region in cell line SVR11/Ad12-C8 is comparable with the E1A expression in control cells transformed by wild-type Ad12E1, but much higher than in cell line SVR11/Ad12-C6. Immunoprecipitation of [^{35}S]methionine-labeled cell extracts (Figure 6) showed that C8 cells, which express high levels of wild-type E1A RNA, contain the same amount of immunoprecipitable E1A protein as the wild-type Ad12 E1-transformed cells but that no detectable E1A protein could be precipitated from C6 cells. Interestingly, immunoprecipitation with a rat alloantiserum showed that class I proteins were drastically decreased in C8 cells, as compared with normal SVR11 cells and C6 cells.

This experiment shows that the expression of class I antigens in class I-positive cells can be switched off following the introduction of a wild-type Ad12 region E1A, but only when this E1A region is expressed at a sufficiently high level. On the basis of this result we tentatively conclude that the low level of class I expression in Ad12-transformed primary cell cultures is caused by an active (*trans*-acting) switching-off process mediated by products of the Ad12E1A region.

Discussion

Primary cells transformed by Ad12E1 have reduced levels of expression of class I MHC transplantation antigens. This reduction was shown to occur at the level of cytoplasmic mRNA and correlates with expression of the 13S mRNA transcribed from the Ad12E1A region (Schrier *et al.*, 1983).

The major question we wanted to answer in this study was whether the low class I expression in Ad12-transformed cells is caused by an active switching-off mediated by Ad12E1A products or is the result of selective transformation by Ad12E1 of cells incapable of expressing normal amounts of class I antigens. Evidence supporting the switching-off mechanism was provided by the experiment in which a wild-type Ad12E1 plasmid was transfected into the class I-positive SVR11-transformed cells. Furthermore, the reduction was shown to occur for all class I loci.

There are several examples of genes, viral as well as non-viral, which are activated in *trans* by Ad5 (or Ad2), and Ad12E1A (Jones and Shenk, 1979; Green *et al.*, 1983; Imperiale *et al.*, 1983; Treisman *et al.*, 1983; Bos and Ten Wolde-Kraamwinkel, 1983; Gaynor *et al.*, 1984). A possible mechanism by which E1A products could regulate gene activity is to modulate the efficiency of formation of transcription complexes (Gaynor and Berk,

1983). For some E1A-regulated genes the sequences involved in their regulation have been shown to be located in the upstream region of these genes (Imperiale *et al.*, 1985; Bos and Ten Wolde-Kraamwinkel, 1983). Furthermore, nuclear run-on experiments have shown that transcription of some E1A regulated viral genes is increased in the presence of E1A (Leff *et al.*, 1984). On the other hand, however, it has been reported that Ad2E1A and Ad5E1A can reduce the activity of the SV40 early enhancer (Borrelli *et al.*, 1984; Velcich and Ziff, 1985). Thus, it seems that Ad5E1A can either activate or suppress gene activity, possibly depending on the promoter region or enhancer of the gene studied. Therefore, it is not suprising that Ad12E1A is capable of both activating the Ad12E1B promoter (Bos and Ten Wolde-Kraamwinkel, 1983) and suppressing class I gene expression.

It is conceivable that different promoter regions are recognized by (slightly) different polymerase II transcription complexes, and that the activity of these complexes is affected differently by E1A products. It is not yet clear, however, whether the difference in class I expression between Ad5- and Ad12-transformed cells occurs at the level of transcription initiation or mRNA stability.

An interesting observation is that the E1A products of Ad5 and Ad12 act differently, i.e. the Ad5E1A products can counteract the action of Ad12E1A, resulting in restoration of class I expression. This dominant effect of Ad5E1A products is most likely the result of competition with Ad12E1A products for binding to the same cellular target involved in the regulation of class I gene expression. This concept is supported by the notion that both the suppressing and the activating effects on class I gene expression of Ad12E1A and Ad5E1A, respectively, are encoded by the first exons of the 13S mRNAs (Jochemsen *et al.*, 1984; Bernards *et al.*, 1983).

The question of why introduction of Ad12E1A into most established cell lines does not result in a reduction of class I gene expression although the expression level of the Ad12E1A region was normal has not yet been answered. It has been suggested that immortalized cells (e.g. HeLa cells) contain cellular Ad5E1A-like factors (Nevins *et al.*, 1984; Kao *et al.*, 1985), and this could explain the failure of Ad12E1A to suppress class I gene activity. However, suppression in immortalized cells was observed when Ad12E1 was introduced into cells transformed by a mutant Ad12E1 plasmid, SVR11. One would have to assume then, that the SVR11-transformed cells do not contain such a factor. Another explanation could be that the SVR11-transformed cells have been immortalized a relatively short while ago, compared with the other immortalized cell lines used. Long-term tissue culture could result in cellular modifications that prevent the class I-inactivating effect of Ad12E1A.

Finally, the results presented in this study seem to contradict those of Rosenthal *et al.* (1985), who showed that infection of mouse embryo cultures with Ad5 as well as with Ad12 results in strong stimulation of class I MHC mRNA expression. Their observation has been confirmed (B.Oostra, personal communication), but we have no explanation for the apparently opposite effects on class I gene expression of Ad12 in transformation and lytic infection.

Materials and methods

All DNA transfections were performed as described by Van der Eb and Graham (1980). Primary cultures of BMK, REB and HER cells were transfected with either pAd5XhoIC (Bernards *et al.*, 1982) or pAd12RIC (Bos *et al.*, 1981) recombinant plasmids containing the E1 regions of Ad5 and Ad12, respectively. 3Y1 cells were co-transfected with either pAd5XhoIC or pAd12RIC and pSV2neo (Southern and Berg, 1983), and BRLtk$^-$ and Ltk$^-$ cells with either pAd5XhoIC or pAd12RIC and a plasmid containing the HSV tk gene (Bos *et al.*, 1983).

FACS analysis was performed on a Ortho Diagnostics System 50-HH and computer MP-2150.

Standard procedures were used for cell labeling with [^{35}S]methionine and subsequent immunoprecipitation (Schrier *et al.*, 1983), S1 nuclease analysis (Jochemsen *et al.*, 1984), isolation of total cytoplasmic RNA and Northern blotting analysis (Schrier *et al.*, 1983).

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