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EXPRESSION OF MHC GENES IN ADENOVIRUS-TRANSFORMED CELLS

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ABSTRACT

Class I MHC gene expression is drastically reduced in Ad12-, but not in Ad5-transformed primary cells of three different rodent species. Here we demonstrate that class I expression in Ad12-transformed primary Baby Rat Kidney cells can be reactivated by Ad5.

We also show that class I gene expression is switched off only when primary cells were transformed by Ad12, but not when established cell lines were transformed.

INTRODUCTION

Human adenoviruses are classified in non-oncogenic and oncogenic species on the basis of their ability to induce tumors in newborn hamsters (1). Adenovirus type 5 (Ad5) is an example of a non-oncogenic species, and Ad12 an example of a highly oncogenic species. Both Ad5 and Ad12, however, are able to transform a variety of cell types in vitro, and the transformed cells often display differences in oncogenicity, i.e. cells transformed by Ad5 are non-oncogenic when injected into syngeneic animals whereas cells transformed by Ad12 are highly oncogenic (2,3).

The transforming region of both viruses has been mapped in the leftmost 11% of the viral genome which contains early region 1 (E1), one of the four regions expressed early during lytic infection (4). Region E1 consists of two transcriptional units, E1a and E1b, which each encode a number of partially overlapping RNAs (5,6). Although the E1 regions of Ad5 and Ad12 show striking similarities, both in structure (7,8) and in functional properties (9), they clearly differ in their ability to induce the oncogenic phenotype.

Studies by Bernards et al. (10) on the oncogenicity of various Ad-transformed cell lines have shown that baby rat kidney (BRK) cells expressing the E1a region of Ad12, in contrast to cells expressing the E1a region of Ad5, are capable of inducing tumors both in nude mice and immunocompetent syngeneic rats. This suggested that cells expressing Ad12 E1a are capable of escaping from the cellular immune response of the rats while cells expressing Ad5 E1a cannot. Schrier et al. (11) have explained these in vivo oncogenicity studies in molecular terms by showing that the level of expression of the heavy chain of the class I transplantation antigens (termed RT1.A for the rat) is drastically reduced in Ad12-transformed primary BRK cells as compared to Ad5-transformed BRK cells. From these data we concluded that Ad12, but not Ad5, is capable of switching off the expression of RT1.A genes in BRK cells. The regulation of RT1.A expression was shown to occur at the level of production of mature mRNA, and to be mediated by the product of the 13S mRNA transcribed from Ad12 E1a.

It is well established that class I transplantation molecules, encoded by the Major Histocompatibility Complex (MHC), play a crucial part in the recognition of (viral) antigens by cytotoxic T-lymphocytes (CTLs), a process known as MHC-restricted recognition of antigen (12). Therefore, one would expect that cells expressing drastically reduced levels of MHC class I antigens will be poorly recognized by CTLs. Using secondary CTL-cultures directed mainly against class I antigens, Bernards et al. (10) have shown that Ad12-transformed BRK cells are indeed poor targets for CTLs, whereas the same CTLs efficiently lysed Ad5-transformed BRK cells. Therefore, we concluded that the difference in oncogenicity between Ad5- and Ad12-transformed BRK cells in immunocompetent rats is caused by the reduced expression of RT1.A genes in the latter.

RESULTS

Is class I gene expression switched off by Ad12 in other species than the rat?

Having shown that rat cells harboring Ad12 E1a have greatly reduced expression of RT1.A antigens, it appeared of interest to examine whether the same differences in expression of class I MHC antigens occur between Ad5- and

Ad12-transformed cells from other species. To answer this question, we transfected primary Balb/c Baby Mouse Kidney (BMK) cells with either pAd5XhoIC (9) or pAd12RIC (7), plasmids containing the entire transforming E1 regions of Ad5 and Ad12 respectively. Foci of transformed cells, which became visible 2 (Ad5) to 3 (Ad12) weeks after transfection, were picked and established as cell lines. All the cell lines tested expressed the adenoviral E1 products as judged by Northern blotting analysis and immunoprecipitation of ^{35}S-methionine-labeled cell extracts (data not shown). The level of class I gene expression in these cells was measured by Northern blot analysis of total cytoplasmic RNA, using a nick-translated human HLA-B7 cDNA clone (13) as a probe. Similar experiments were also performed with Ad5- and Ad12-transformed Hamster Embryo cells. The results of these experiments, which are summarized in Table 1, show that in the three rodent species tested (rat, mouse, hamster) the expression of class I MHC genes is strongly decreased in Ad12-, but not in Ad5-transformed primary cells.

Table 1

Expression of MHC class I genes in adenovirus-transformed cells.

1. Primary cells	Transformed by	MHC expression
Baby Rat Kidney	Ad5	+
"	Ad12	-
"	Ad12+myc	-
Baby Mouse Kidney	Ad5	+
"	Ad12	-
Hamster Embryo	Ad5	+
"	Ad12	-
2. Cell lines		
Rat 3Y1	Ad5	+
"	Ad12	+
Rat BRLtk$^-$	Ad5	+
"	Ad12	+
Mouse Ltk$^-$	Ad5	+
"	Ad12	+

In the hybridization experiments described thusfar a cDNA copy of a human MHC gene (HLA-B7) was used to determine expression of MHC genes in adeno-transformed cells (11, see also Fig.2). We have also tested the expression of MHC genes in our Ad-transformed cells by using a cDNA clone of a mouse H2 gene of the d-haplotype, kindly provided by Kourilsky (14). This cDNA clone pH2^{d}-1, derived from the H2D^{d} gene (Kourilsky, personal communication) is almost identical to pAG64. Brickell et al.(15) have suggested that pAG64 is a cDNA copy of one of the genes of the Qa/T1a complex.

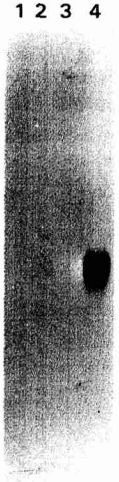

Figure 1. Norther analysis of class I MHC transcripts hybridizing to pH2^{d}-1 in untransformed and Ad-transformed BRK cells. Electrophoresis, blotting and hybridization conditions were as described previously (10). 20 µg total cytoplasmic RNA (lane 1) or 1 µg poly(A)$^{+}$ RNA (lanes 2,3,4) were applied to the gel.
Lanes 1 and 3: Ad12-transformed BRK cells,
Lane 2: untransformed BRK cells,
Lane 4: Ad5-transformed BRK cells.

Fig.1 shows that the cDNA clone pH2^{d}-1 hybridizes to a transcript of 1.6 kD to a much higher level with RNA from Ad5-transformed BRK cells (lane 4) than from untransformed BRK cells (lane 2). Therefore, our results seem to confirm those of Brickell et al (15) that elevated levels of transcription of MHC genes are found in transformed cells. Fig.1, lanes 1 and 3, however, shows that pH2^{d}-1 detects no transcripts in Ad12-transformed BRK cells. Similar results were found in BMK cells transformed by Ad5 and Ad12 (data not shown). The low level of expression in Ad12-transformed cells could be explained by a suppression of transcription by an E1 gene product. Thus, the results obtained with the human HLA-B7 clone are confirmed by using the mouse pH2^{d}-1 clone.

Is reduced class I expression caused by active switching off?

We have also examined whether the low expression of RT1.A in Ad12-transformed BRK cells is indeed caused by an inhibition of class I gene expression mediated by the gene product of the 13S E1a mRNA of Ad12, or whether Ad12 selectively only transforms cells in the BRK population that are intrinsically incapable of RT1.A expression. The latter possibility could not be excluded, as the primary BRK cultures used for transformation are possibly heterogeneous in class I gene expression and because the transformation efficiency of Ad12 is 10-20 fold lower on these cells than that of Ad5 (9).

To distinguish between the possibilities mentioned above we have used established cell lines for transformation experiments, because these are considered to be more homogeneous than primary cell cultures. Rat cell line 3Y1 (16) was co-transfected with either pAd5XhoIC or pAd12RIC and pSV2 neo, a plasmid conferring resistance against the kanamycin-analog G-418 (17). Resistant colonies were picked 2-3 weeks after transfection and grown in mass culture. Northern blotting analysis revealed that although the adenovirus E1 genes were transcribed no difference in RT1.A expression occurred between 3Y1 cells transformed by Ad5 and Ad12 (Figure 2). Identical results were obtained with the

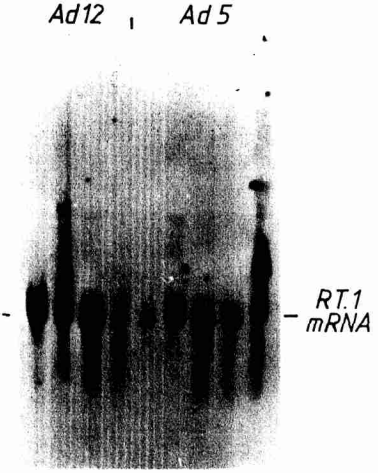

Figure 2. Analysis of class I MHC transcripts in rat cell line 3Y1 transformed by Ad5 or Ad12. 20 µg total cytoplasmic RNA was applied to a 1% agarose-formaldehyde gel. A nick-translated human HLA-B7 cDNA clone (13) was used as probe.

rat cell line BRLtk$^-$ and with mouse Ltk$^-$ cells (summarized in Table 1). This observation suggested that, if Ad12 region E1a has the ability to turn off expression of class I genes, it is apparently not able to do so when it transforms established cell lines. The question whether Ad12 E1a can switch off class I gene expression can, therefore, not be answered by use of extablished cell lines.

To further examine the question whether the reduced expression of class I genes in Ad12-transformed BRK cells is due to a switching off process (rather than to selection of cells expressing low levels of class I genes) advantage was taken from a previous observation showing that primary BRK cells transformed by Ad12 region E1 as well as by Ad5 region E1a express normal levels of RT1.A (10), suggesting that Ad5 E1a can restore class I gene expression, or counteract the suppressing effect of Ad12 E1a.

To investigate whether RT1.A expression can be reactivated in RT1.A-negative cells, Ad12-transformed BRK cells were co-transfected with pAd5XhoIC and pSV2 gpt (18). Mycophenolic acid-resistant colonies were established as cell lines and tested for their expression of the transfected Ad5 E1 region and RT1.A by immunoprecipitation of ^{35}S-methionine-labeled cell extracts. Apart from the Ad5-encoded proteins all cell lines expressed RT1.A in amounts comparable to the level expressed in Ad5-transformed cells (data not shown). We feel that this result strongly argues against the explanation that the reduced level of RT1.A expression in Ad12-transformed BRK cells is caused by the selective transformation of primary BRK cells incapable of expressing RT1.A. Instead it strengthens our hypothesis that the product of the 13S mRNA transcribed from Ad12 E1a causes the inactivation of RT1.A gene expression, and that the Ad5 E1a products can restore RT1.A expression possibly through competition with Ad12 E1a products for binding to the same regulatory factors.

Since it was recently reported that the Ad5 E1a region shows a structural homology with the *myc* oncogen (19) we investigated whether the *myc*-product could also prevent the reduction of RT1.A expression in Ad12-transformed BRK cells. Primary cultures of BRK cells were co-transfected with pAd12RIC and a human LTR-activated c-*myc* gene (kindley provided by Dr.Stehelin). Foci of Ad12-transformed BRK cells are normally observed approximately 4 weeks after transfection; in this particular experiment, however, foci were already seen as early as 10 days after transfection. These

foci were isolated, established as cell lines, and expression of the transfected myc gene was confirmed by Northern blot analysis of total cytoplasmic RNA (data not shown). Immunoprecipitations of ^{35}S-methionine-labeled cell extracts revealed that RT1.A expression was as low in myc-expressing Ad12-transformed BRK cells as in cells transformed by Ad12 alone (Table 1), indicating that an (overexpressed) myc-product by itself does not reactivate RT1.A expression in Ad12-transformed BRK cells.

Class II expression

The question can be asked whether the oncogenicity of Ad12-transformed primary BRK cells in immunocompetent rats can be solely explained by the fact that they do not elicit a class I MHC-restricted immune response, or whether other immunological responses, such as NK cell activity or class II MHC-restricted responses are also impaired?

It has been previously described that rat kidney endothelial cells can express class II MHC molecules (20). Therefore, it seemed of interest to investigate whether Ad5-transformed BRK cells also expressed these class II MHC molecules, and if so, whether their expression was reduced in Ad12-transformed BRK cells. To address this question Ad5- and Ad12-transformed primary BRK cells were surface labeled with ^{125}I-iodine, using lactoperoxidase, and cell extracts were immunoprecipitated with a mouse anti-rat class II-specific antiserum (kindly provided by Dr.L. Vaessen). Rat spleen cells, stimulated with the B-cell mitogen Poke Weed Mitogen, were used as positive control as B-lymphocytes are known to express large amounts of class II molecules. Figure 3 shows that the anti-class I antiserum recognizes more class II molecules from ^{125}I-iodine labeled spleen cells than the anti-class II antiserum itself. Both anti-sera, however, failed to precipitate any class II molecules from both Ad5- and Ad12-transformed BRK cells, suggesting that class II antigens on Ad-transformed BRK cells play no part in the immunological recognition of these cells in vivo.

DISCUSSION

In the present study we have shown that the level of class I MHC gene expression is not only strongly reduced in rat kidney cells transformed by Ad12 but also in Ad12-

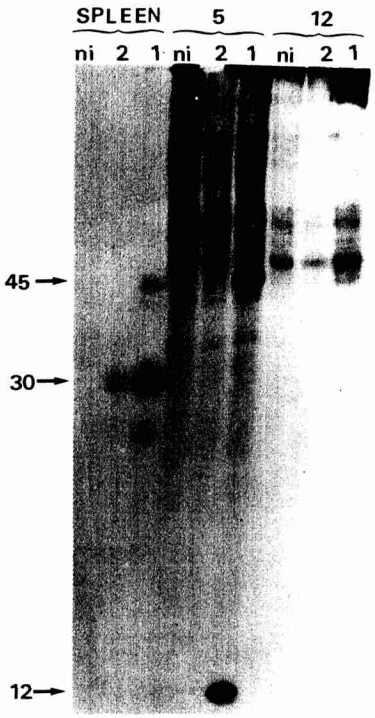

Figure 3. SDS-polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of ^{125}I-labeled rat spleen cells and Ad5- and Ad12-transformed BRK cells with normal mouse serum (n.i.), mouse anti-rat class II antiserum (2), and Lewis anti-Wag-Rij class I alloantiserum (1).

transformed cells derived from two other rodent species, mouse and hamster. We have previously shown that the reduction of class I gene expression occurs at the level of production of mature mRNA, and is caused by the product of the 13S mRNA transcribed from Ad12 region E1a (11).

To investigate whether cells that have a very low level of class I gene expression as a result of transformation by Ad12 are intrinsically capable of expressing these MHC genes, we have supertransformed RT1.A-negative Ad12-transformed BRK cells with Ad5 region E1 (we have previously shown that primary BRK cells transformed by region E1 of both Ad5 and Ad12 express class I genes normally, indicating that the

suppressing effect of Ad12 E1a is recessive in the presence of Ad5 E1a (10)).

Here we show that class I gene expression in Ad12-transformed cells is fully restored upond supertransformation with Ad5, indicating that Ad12-transformed BRK cells are potentially able to express RT1.A. This observation strengthens our hypothesis that the reduced level of class I expression in Ad12-transformed BRK cells is caused by an active switching-off process. In contrast to primary cells transformed by Ad12, class I expression is not reduced in cells that were transformed by Ad12 when they were already established as cell lines (Table 1, Figure 2). Apparently, a factor that is present in cell lines but not in primary cells, prevents that class I gene expression is switched off by Ad12. The nature of this factor is unknown, but it could be involved in immortalization. As Ad5 E1a also prevents the inactivation of class I expression in Ad12-transformed BRK cells (10),and Ad5 E1a has been reported to be structurally related to the myc-oncogene (19), we investigated whether enhanced expression of myc also prevents the reduction of class I expression in Ad12-transformed primary cells. Introduction of a human LTR-activated c-myc gene (kindly provided by Dr.Stehelin) in Ad12-transformed cells, however, did not cause activation of class I genes, suggesting that the myc product by itself in this respect does not show functional homology with Ad5 E1a. It cannot be excluded, however, that this negative result is due to the fact that the human c-myc gene does not function properly in rat cells.

It was recently reported by Brickell et al. (15) that another class I MHC gene which they claimed to be encoded by the Qa/T1a locus, is specifically activated in transformed cells and tumor cells. Using a cDNA probe complementary to the H-2D^{d} mRNA (P. Kourilsky, personal communication) which is almost identical to the one used by Brickell et al., we show here that the level of hybridizing transcripts is strongly enhanced in Ad5-transformed BRK cells as compared to untransformed BRK cells (Figure 1). Again, like the HLA-B7 cDNA, pH2^{d}-1 detects no transcripts in Ad12-transformed BRK cells. Thus, the results obtained with the two different class I cDNA probes are similar for Ad-transformed BRK cells; a different level of expression is detected in untransformed BRK cells.

Schrier et al.(11) have shown that a protein with an apparent molecular weight of 32kD is present in untrans-

formed and Ad5-transformed BRK cells, but absent from Ad12-transformed cells. We show here that this 32kD protein does not appear to be a class II MHC molecule since this category of MHC genes is neither expressed in Ad5-, nor in Ad12-transformed BRK cells (Figure 3). Circumstantial evidence, however, suggests that this 32kD protein may be another MHC-related protein since a 32kD protein co-precipitates with β_2-microglobulin immunoprecipitated with anti-β_2m antiserum in untransformed and Ad5-transformed cells, but not in Ad12-transformed cells (R.Vaessen, unpublished observations).

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