Evidence for the Expression of Protein IX in Some Rat Cells Transformed with Adenovirus Type 12 Early Region 1 DNA

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(Accepted 15 June 1987)

SUMMARY

Sera were produced by injection of a particular rat cell line (HLBRK EcoC1), produced by transformation of baby rat kidney cells with adenovirus 12 early region 1 DNA, into the syngeneic host. Antibodies to a protein of Mr 15200 as determined by SDS-PAGE, were detected in the sera from two rats bearing tumours. The 15K protein was identified as the viral structural component polypeptide IX by a number of criteria: (i) the protein was only observed in adenovirus 12-infected cells, (ii) it was produced at intermediate and late times after infection, (iii) it was immunoprecipitated from purified adenovirus 12 particles and (iv) it was immunoprecipitated from a purified protein preparation.

The first proteins expressed after the onset of adenovirus infection are encoded by the portion of DNA located at the left-hand end of the viral genome (for review, see Bernards & van der Eb, 1984; Branton et al., 1985; Gallimore et al., 1985a; Grand, 1987). This area, termed early region 1 (E1), has been divided into two transcription blocks, E1A and E1B (Berk & Sharp, 1978). At early times after adenovirus 12 (Ad 12) infection the former of these encodes a family of proteins of Mr about 41000 (41K), whilst the latter encodes two major polypeptides of Mr 19K and 54K, as determined by SDS-PAGE.

Transfection of primary human and rodent cells grown in culture with this same region of adenovirus DNA results in the production of foci which can be passaged into immortal, transformed cell lines. A 'fully transformed' phenotype is exhibited by those cells expressing the E1A and E1B polypeptides whereas a 'partially transformed' phenotype is apparent with those cells expressing either E1A alone, or E1A and the smaller E1B protein (Shiroki et al., 1979; Jochemsen et al., 1982; Gallimore et al., 1985b).

A further major polypeptide encoded by the E1B DNA is translated from a 9S mRNA and is generally expressed both before the onset of viral replication and at late times in infection. This protein, designated polypeptide IX, is a virion structural component and has not been detected in cells transformed by either adenovirus E1 DNA or with Ad 12. Previously this polypeptide has only been observed after translation in vitro of mRNAs prepared from adenovirus-transformed cells (for example, see Lewis & Matthews, 1981). However, in the report presented here we show that on occasion either transient or very low level expression of protein IX may occur in rat cells transformed with the Ad 12 EcoC DNA fragment (encompassing the whole of the E1 region), when they are injected into the syngeneic host, as judged by the production of specific high titre polyclonal antisera.

Human embryo kidney (HEK) cells were infected with Ad 12 at a m.o.i. of 60 p.f.u./cell. Cells, radiolabelled with [35S]methionine for 2 h, were harvested at appropriate times after infection, suspended in lysis buffer and subjected to immunoprecipitation using the method essentially as described by Paraskeva et al. (1982). The tumour bearer sera (TBS) used in these experiments were produced in rats injected with either an Ad 12 HLBRK HindIIIIG-transformed rat cell line
Fig. 1. Immunoprecipitation of Ad 12 proteins following infection of HEKs. Cells were incubated for 1 h in methionine-free medium, labelled with $^{35}$S-methionine (200 μCi/10 cm dish of HEKs) for 2 h and harvested at the times indicated following Ad 12 infection. Samples were processed and immunoprecipitated as described by Paraskeva et al. (1982) using the following antibodies: lane 1, TBS from a rat injected with Ad 12 HLBRK EcoC1 cells; lane 2, antibodies to Ad 12 structural proteins; lane 3, TBS from a rat injected with Ad 12 HLBRK HindIIIG cells. Molecular weights of the Ad 12 E1 proteins are indicated.

(containing antibodies to the Ad 12 E1A and smaller Ad 12 E1B polypeptides), with the Ad 12 HLBRK EcoC3 cell line (containing antibodies to the three major E1 proteins) or with the Ad 12 HLBRK EcoC1 cell line (the EcoC1 and EcoC3 cell lines were the products of unique foci formed after transfection of HLBRK cells with a plasmid containing Ad 12 E1 DNA; Gallimore et al., 1985b). A polyclonal antiserum containing antibodies to the major Ad 12 structural proteins was also used (this serum was raised against SDS-disrupted Ad 12 virions). It can be seen from Fig. 1 that the E1A 41K proteins could be detected at about 12 h post-infection and the E1B polypeptides at about 18 h post-infection. At the same time a protein of $M_r$ 15200 was immunoprecipitated, with the Ad 12 HLBRK EcoC1 TBS. At slightly later times (21 h) adenovirus major structural proteins became apparent.

The protein of $M_r$ 15K detected with the Ad 12 HLBRK EcoC1 TBS was potentially a previously uncharacterized tumour antigen. It was, however, only immunoprecipitated with one other rat TBS out of a panel of 21 tested (data not shown). This positive serum had also been produced by the injection of the same Ad 12 HLBRK EcoC1 cell line. The 15K-negative TBS
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Fig. 2. Immunoprecipitation of Ad 12 proteins following infection with Ad 12 mutants: (a) H12in600, (b) H12in602 and (c) H12hr703. HEKs were infected with mutant virus (200 p.f.u./cell) for 24 h and labelled with [35S]methionine. The following antisera were used: lane 1, TBS from a rat injected with Ad 12 HLBRK EcoC1 cells; lane 2, TBS from a rat injected with Ad 12 HLBRK HindIIIG cells; lane 3, antibodies to Ad 12 structural proteins. The molecular weights of the Ad 12 E1 proteins are indicated.

tested were from rats injected with either other rat cell lines (produced by transformation with Ad 12 E1A, Ad 12 E1 DNA or with Ad 12) or with Ad 12 virus.

In an attempt to pinpoint the E1 gene encoding the 15K protein similar time courses of infection were set up using a number of Ad 12 mutants containing lesions in the E1 DNA region (Gallimore et al., 1986; Grand & Gallimore, 1986). It was observed that the 15K protein was strongly expressed following infection with H12in600 (an E1A mutant that does not express the protein product of the E1A 13S mRNA but does express the product of the 12S mRNA; since these two polypeptides comigrate under the conditions used here the protein pattern on the gel appeared similar to wild-type) (Fig. 2) and with H12 m14-2 which does not express the E1B 19K protein (data not shown). With those mutant viruses (hr703 and in602) which fail to express the larger E1B protein (Grand & Gallimore, 1986), however, no 15K polypeptide can be seen. Although this initially suggested that it is translated from the same 22S mRNA as the 54K protein, it became apparent, particularly in the light of the data presented below, that the absence of the 15K protein from these infections is due to the lack of viral replication and hence viral structural proteins rather than the lack of transcription from E1B DNA.

The presence of antibodies to the 15K protein in TBS was obvious evidence of expression of that polypeptide in the transformed cells injected into the syngeneic host. Therefore, two other approaches, based on this fact, were adopted in an attempt to identify the polypeptide unequivocally. First, lysates of the cell line Ad 12 HLBRK EcoC1 and of other Ad 12-transformed rat and human cells were subjected to immunoprecipitation using the HLBRK EcoC1 and the HindIIIG antisera; only the major E1A (41K) and E1B (19K and 54K) polypeptides could be detected (Fig. 3a). Second, using the Western blotting procedure described by Grand & Gallimore (1984) tumours obtained from rats injected with the HLBRK EcoC1 cell line were examined for the presence of the 15K protein. Tissue extracts were prepared by homogenizing 1 g of tumour in 10 ml of 9 M-urea, 50 mM-Tris–HCl pH 7.5, 15 mM-2-mercaptoethanol; insoluble material was removed by centrifugation. Aliquots (10 μl) were subjected to electrophoresis either in the presence of SDS or of 7 M-urea (Grand & Gallimore, 1984), the proteins were transferred to nitrocellulose filters and these were probed with the Ad 12 HLBRK EcoC1 or Ad 12 HLBRK HindIIIG antisera; in neither case could any protein be detected which corresponded to the 15K polypeptide observed in infected cells.

To establish that the 15K protein was of viral rather than cellular origin and to confront the possibility that it might be a structural protein rather than a conventional E1 tumour antigen, purified Ad 12 particles were disrupted with 1% SDS and radiolabelled with 125I using the
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Fig. 3. (a) Immunoprecipitation with the Ad 12 HLBRK EcoC1 TBS. [35S]methionine-labelled cell extracts were immunoprecipitated with the Ad 12 HLBRK EcoC1 TBS as described in the text. The following cells were used: lane 1, human embryo retinal cells transformed with Ad 12 E1 DNA; lane 2, Ad 12 HLBRK EcoC1 cells; lane 3, Ad 12 HLBRK HindIIIIG cells; lane 4, Ad 12-infected HEKs (24 h post-infection). The molecular weights of the Ad 12 E1 proteins are indicated. (b) Immunoprecipitation of [125I]labelled Ad 12. Adenovirus 12 disrupted with SDS was labelled with 125I as described in the text and subjected to immunoprecipitation using the following antisera: lane 1, normal rat serum; lane 2, TBS from a rat injected with Ad 12 HLBRK HindIIIIG cells; lane 3, TBS from a rat injected with Ad 12 HLBRK EcoC3 cells; lane 4, antibodies to Ad 12 structural proteins; lane 5, TBS from a rat injected with Ad 12 HLBRK EcoC1 cells. Molecular weight markers are indicated. (c) Immunoprecipitation of 125I-labelled protein IX. Protein IX was purified and labelled as described in the text and subjected to immunoprecipitation. Lane 1, 125I-labelled purified protein IX; lanes 2 to 5, immunoprecipitation of labelled protein using the following antisera: lane 2, TBS from a rat injected with Ad 12 HLBRK EcoC3 cells; lane 3, TBS from a rat injected with Ad 12 HLBRK HindIIIIG cells; lane 4, TBS from a rat injected with Ad 12 HLBRK EcoC1 cells; lane 5, antibodies to Ad 12 structural proteins. Molecular weight markers are indicated.

chloramine T procedure. Viral proteins were separated from unbound 125I and SDS by chromatography on a column of Sephadex G-25 eluted with 0-2 m-Tris–HCl pH 7-5 containing 2% (w/v) bovine serum albumin and subjected to immunoprecipitation by the method of Paraskeva et al. (1982). It can be seen from Fig. 3(b) that a protein of Mr 15K was efficiently precipitated from the 125I-labelled virus extract with the HLBRK EcoC1 serum, but not with other comparable TBS. Small amounts of the protein could be seen on over-exposed autoradiographs after immunoprecipitation with the antibody directed against the viral structural proteins.

On the basis of these observations we concluded that the 15K polypeptide was probably Ad 12 protein IX. As a final confirmation of this hypothesis protein IX was isolated from caesium chloride-purified Ad 12 particles using the method of Everitt & Philipson (1974). The polypeptide was labelled with 125I using the chloramine T method as described above, and subjected to immunoprecipitation with the HLBRK EcoC1, HLBRK EcoC3, HindIIIIG and viral structural protein antibodies. It can be seen from Fig. 3(c) that 125I-labelled protein IX was precipitated very well with the EcoC1 TBS, poorly with structural protein serum and not at all with the HindIIIIG and EcoC3 TBS. It is reasonable to suppose therefore that on rare occasions the structural polypeptide can be expressed in tumours formed after the injection of rat cells, transformed with Ad 12 E1 DNA, into the syngeneic host. The explanation for the relative inefficiency of the viral structural protein antiserum in the immunoprecipitation experiments shown in Fig. 1, 2 and 3 probably lies in its low titre due to the small amount of polypeptide IX in the virus particles. It should be noted however that the protein must be relatively antigenic since the high titre EcoC1 antibody seems to have been produced during a limited exposure of the host animal. However, whether this exposure was transiently at a high level or over a prolonged period at a very low level (i.e. not detected using the procedures described above) is not clear at present.

Polypeptide IX is a protein of 144 amino acids and Mr 15000 (Bos et al., 1981). It is a minor constituent of the virus particle, but is expressed at intermediate and late times following viral infection i.e. both before and after the onset of viral replication (Persson et al., 1978) as has been
observed here. Expression of the protein is not considered to be essential since the adenovirus 5 dl313 mutant (Colby & Shenk, 1981) which lacks the gene is still viable. The DNA encoding polypeptide IX is contained within the E1 gene block and can be transcribed to give a 9S mRNA. The protein has, however, never before been unequivocally demonstrated within cell lines produced after transformation with adenovirus E1 DNA. It seems likely that mRNAs, encoding E1B polypeptides of Mr 10K to 20K observed after adenovirus infection are genuine early products (Katze et al., 1982; Anderson et al., 1984; Virtanen & Pettersson, 1985; Mak & Mak, 1986) as opposed to the structural protein reported here. Obviously, expression of protein IX is not necessary for the acquisition or maintenance of the transformed phenotype; indeed it might be supposed that the presence of protein IX confers a definite growth disadvantage on those cells which express the polypeptide since no cell lines have been isolated in which it is constitutively and continuously expressed. Examination of a very large number of Ad 12-transformed cell lines and Ad 12-derived TBS has not, in the past, demonstrated the presence of the protein (for example, see Gallimore et al., 1985b).

We are most grateful to Professor Jim Williams, University of Pittsburgh, Pa., U.S.A. for a gift of the Ad 12 mutant virus H12 m14-2. We also wish to thank Ann Maguire, Val Nash and Liz Fletcher for valuable technical assistance, Debbie Williams for excellent secretarial help and the Cancer Research Campaign, London for financial support. P.H.G. is a CRC Life Fellow.

REFERENCES


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(Received 11 May 1987)