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Expression of Hamster MHC Class I Antigens in Transformed Cells and Tumours Induced by Human Adenoviruses

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Abstract—*The steady-state levels of hamster MHC class I mRNA and cell surface protein were analysed in cells transformed by either adenovirus type* 12 (*Ad*12) *or types* 2 *or* 5 (*Ad*2 *or Ad*5). *All cell lines were oncogenic in new born and adult hamsters. A great reduction in both class I mRNA and protein was observed in Ad*12 *transformed cells compared to cells transformed by Ad*2 *or Ad*5. *Analysis of class I mRNA in solid tumours induced in hamsters by Ad transformed cell lines also showed greatly reduced mRNA levels in tumours induced by Ad*12 *compared to those induced by Ad*2 *or Ad*5. *This suggests that, in the Ad transformed hamster cell system, reduction in the level of MHC class I gene expression is not necessarily associated with tumour formation.*

INTRODUCTION

HUMAN ADENOVIRUSES (Ads) can be divided into subgroups on the basis of their oncogenicity in newborn rodents [1]. Their oncogenic potential ranges from the highly oncogenic subgroup A (e.g. Ad12), which induces tumours at high frequency with a short latency period, to the non-oncogenic subgroup C (e.g. Ad2, Ad5). Cultured rodent cells, however, can be transformed by virions or isolated DNA of all serotypes [2]. Studies on transformation mediated by DNA fragments generated by restriction endonucleases show that the genetic information necessary and sufficient to establish the transformed state is located in the left 11% of the adenovirus genome [3]. Two transcriptional units are present within this transforming region, the first being from 1.5 to 4.5% (E1A) and the second (E1B) from 4.5 to 11.5% [4]. Rat cells transformed by Ad12 are highly oncogenic in both athymic nude mice and syngeneic animals, whereas cells transformed by Ad2 or Ad5 are usually non-oncogenic in syngeneic animals and are also only weakly oncogenic in nude mice [5].

Dissection of the adenovirus transforming region shows that rat cells expressing the Ad5E1A and Ad12E1B genes are non-tumorigenic in newborn rats, whereas cells expressing either the Ad12E1A gene alone or the Ad12E1A and Ad5E1B genes could together form tumours when tested in the same animal system [6]. It is proposed that cytotoxic T lymphocytes (CTLs) may be responsible for rejection of Ad2 and Ad5 transformed cells [5] and that Ad12 transformed cells, which contain reduced levels of surface MHC class I antigens [7] escape immune surveillance by CTLs. Other research proposes that natural killer (NK) cells are responsible for rejection of Ad2 and Ad5 transformed cells and that Ad12E1A gene expression confers resistance to NK cell killing [6, 8].

In this study, we have examined the expression of MHC class I genes in Ad5 and Ad12 transformed hamster cells. The HE1 and H14b cell lines are hamster embryo cells transformed by non-oncogenic Ad2 and Ad5 respectively [9, 10] and HA12/7 is derived from the same cell source by transformation by highly oncogenic Ad12 [11]. All these cell lines are highly tumorigenic in both newborn and adult hamsters. Therefore HE1 and H14b cells present an interesting contrast to Ad2 or Ad5 transformed rat and mouse cells which are usually non-oncogenic in newborn animals [7, 12]. To compare results obtained in this study with adenovirus transformed rat cells, two rat cell lines were examined. RFC-1 is a highly tumorigenic Ad12 transformed rat cell line [13] whereas F19 is an Ad2 transformed rat cell line which is non-tumorigenic in newborn rats [12, 14].

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We show that the decreased expression of MHC class I genes seen in Ad12 transformed hamster cells is not necessarily associated with tumour formation since tumours induced by Ad2 or Ad5 transformed cells contain relatively high levels of class I mRNA. The Ad2 and Ad5 transformed hamster cells also contain class I antigens at their cell surfaces.

MATERIALS AND METHODS

Cells

All cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (Gibco, U.K.).

Tumours

Tumours were induced by sub-cutaneous inoculation of approx. 10^4 viable cells in 0.1 ml PBS into neonatal golden hamsters. After approx. 3 weeks palpable tumours were evident, the animals were sacrificed and tumour tissue was removed, quick frozen in liquid N_2 and stored at −70°C.

Immunochemical methods

Cells were grown on 2 × 2 cm glass coverslips and fixed in 3.7% formaldehyde in PBS for 20 min at room temperature. Following a 20 min rinse in PBS, cell surface labelling was performed by incubation with primary antibody for 60 min at 37°C, washing in PBS and then identical incubation with FITC conjugated second antibody (Miles, U.K.) at 1:50 dilution in PBS. Coverslips were mounted, viewed and photographed in a Leitz Dialux Vario-orthomat system. Primary antibodies used in this study were: monoclonal antibody containing culture supernatants OX18 (anti-rat class I heavy chain, obtained from Serotec, U.K.) and RA1 (anti-adenovirus 2 and 5 E1B 58,000 mol. wt protein, [15]), rabbit anti-human β_2-microglobulin (obtained from Serotec, U.K. and used at 1:50 dilution) and normal mouse serum used at 1:50 dilution.

mRNA analysis

Polyadenylated RNA was isolated from either cells or tumours and analysed by Northern blotting using standard methods [16]. Aliquots (4 μg) of RNA were separated by electrophoresis in 1.5% agarose–2.2 M formaldehyde horizontal gels at 100 V for 5 h and nucleic acids transferred to nitrocellulose filters [16]. Filters were pre-hybridized for 2 h at 42°C in 50% formamide, 1 × Denhardt's solution (1 × Denhardt's solution is 0.2% Ficoll, 0.2% BSA, 0.2% polyvinylpyrrolidone), 5 × SSC (1 × SSC is 15 mM sodium citrate, 150 mM NaCl), 100 $\mu g ml^{-1}$ sonicated salmon sperm DNA and 12.5 μM sodium pyrophosphate. Hybridization was performed at 42°C for 18 h in the same buffer to which was added dextran sulphate (to 5%, w/v) and denatured, nick-translated DNA probe. After hybridization the filters were washed 4 times for 20 min at 42°C in 2 × SSC, 0.1% SDS and once for 30 min at 42°C in 2 × SSC. The filters were dried and exposed to X-ray film (Fuji RX, Dorcan, Wilts, U.K.) for 18 h at −70°C using a phosphotungstate intensifying screen.

DNA probes were either a 1.8 kb PstI fragment of the murine H-2K^b gene [17] or a 1 kb PstI fragment of a mouse β-actin cDNA clone [18]. Labelling was performed by nick-translation [16] using [α-^{32}P]dCTP (New England Nuclear, 3000 Ci/mmole) and the specific activity of the labelling DNA probes was approx. 10^8 dpm/μg).

RESULTS

Analysis of steady-state levels of MHC class I mRNA transcripts by Northern blotting showed that the mouse H-2K^b probe hybridized to an approx. 1.8 kb RNA species from both rat and hamster cells (Fig. 1A). This class I mRNA was readily detected in Ad2 and Ad5 transformed hamster (Fig. 1A lanes 1 and 5) and rat cells (Fig. 1A lane 7) but was markedly decreased in level in both Ad12 transformed hamster and rat cells (Fig. 1A lanes 3 and 8 respectively). Interestingly, in solid tumours taken from hamsters which had been injected shortly after birth with Ad5, Ad12 or Ad2 transformed hamster cells, a pattern of expression of class I mRNA was found which was identical to that of the respective cell lines growing in culture (Fig. 1A lanes 2, 4 and 6 respectively). Therefore, relatively high levels of expression of class I genes were detected in Ad2 and Ad5 transformed cell lines growing as solid tumours *in vivo*.

To ensure that equivalent masses of RNA had been analysed in the Northern blotting experiment, each RNA sample was electrophoretically separated in parallel and the resulting identical nitrocellulose filter was hybridized with a nick-translated mouse β-actin cDNA fragment [18] which identified an approx. 2 kb mRNA species unaltered in level by viral transformation [19]. This control experiment showed that approximately equivalent masses of RNA of each cell line had been analysed by Northern blotting (Fig. 1B).

In order to confirm that *bona fide* tumour tissue had been taken from tumours for RNA analysis, and also that there was no selection for altered forms of the transformed cells in the tumour, a control experiment was performed. A small piece of H14b tumour tissue was trypsinized and cultured *in vitro* for 2 days, after which time the cells were fixed and examined by indirect immunofluorescence using a monoclonal antibody specific for the major 58,000 mol. wt polypeptide specified by the Ad5E1B gene [15]. Each cell in the H14b tumour explant culture

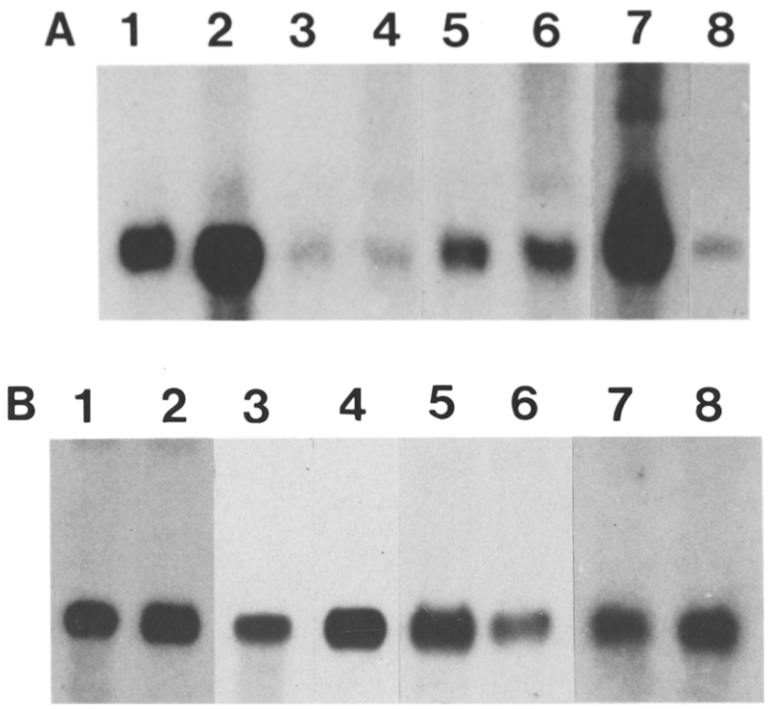

Fig. 1. Northern blotting analysis of polyadenylated RNA. RNA samples (4 μg) were separated by denaturing agarose gel electrophoresis, blotted onto nitrocellulose filters and hybridized with nick-translated H-2K^b (A) or βactin (B) DNA fragments. RNA was isolated from H14b cells, H14b tumour tissue, HA12/7 cells, HA12/7 tumour tissue, HE1 cells, HE1 tumour cells, F19 cells, RFC-1 cells (lanes 1–8 respectively). 28S and 18S ribosomal RNAs were used as size markers.

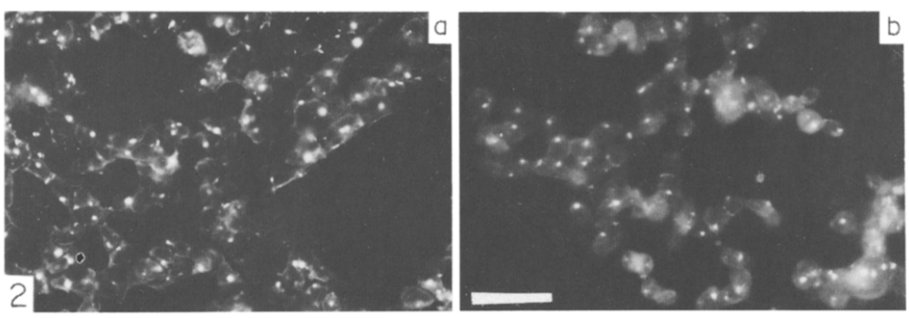

Fig. 2. Indirect immunofluorescent antibody staining of H14b tumour explant (a) and H14b cells grown in culture (b) with monoclonal antibody against adenovirus 5 E1B 58,000 mol. wt protein. Bar = 10 μm.

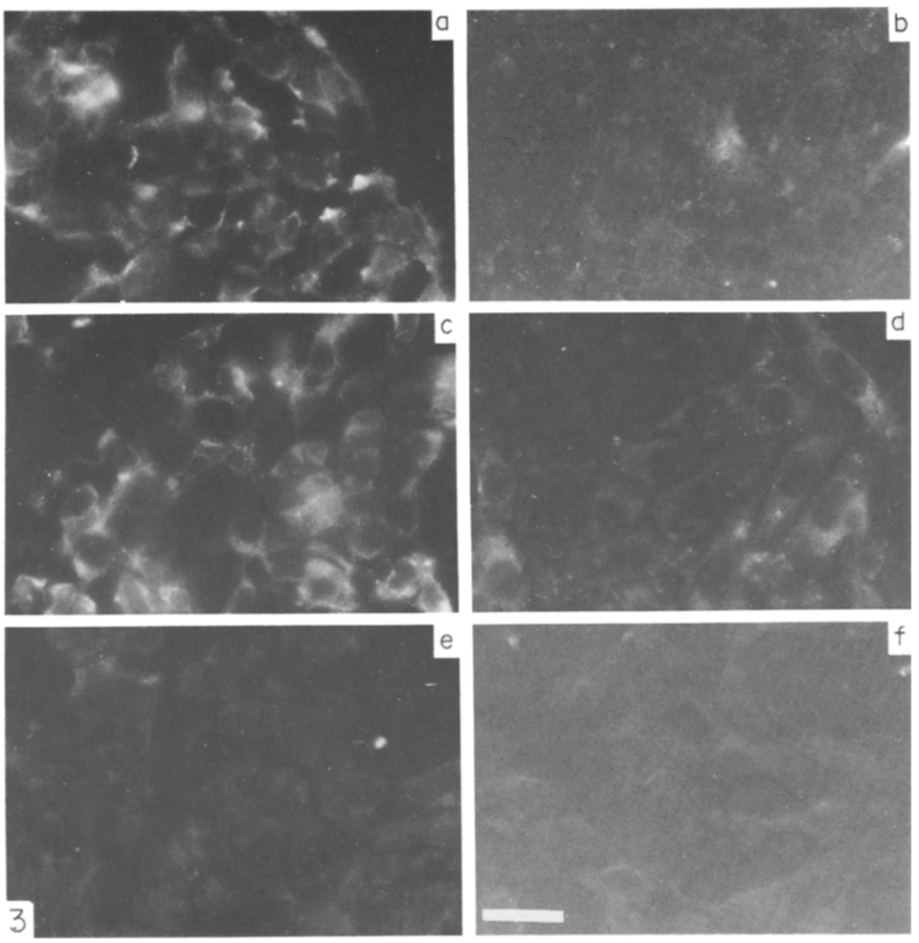

Fig. 3. Surface staining of class I heavy chain and β_2-microglobulin in H14b and HA12/7 cells. H14b cells (a,c,e) and HA12/7 cells (b,d,f) were stained using antibody OX18 against class I heavy chain (a,b), antibody against β_2-microglobulin (c,d) and normal mouse serum (e,f). Bar = 20 μm.

showed specific and characteristic nuclear and perinucler staining by the monoclonal antibody (Fig. 2a), identical to the original H14b culture maintained *in vitro* (Fig. 2b) demonstrating that the tumour tissue sample taken for RNA analysis consisted of Ad5 transformed cells. Similar results were obtained for HE1 cells (not shown).

The presence of class I heavy chain and β_2-microglobulin proteins on the surface of H14b cells was confirmed by indirect immunofluorescence (Fig. 3). Since no monospecific antibody against the hamster class I heavy chain was available, we tested several monoclonal antibodies again rat class I heavy chain for their ability to immunoprecipitate a 45,000 mol. wt polypeptide (previously characterized as the hamster class I heavy chain [20]) from metabolically labelled H14b cells. Monoclonal antibody OX18 precipitated a 45,000 mol. wt protein and polyclonal anti-human β_2-microglobulin precipitated a 12,000 mol. wt protein from these cells (results not shown). Neither polypeptide was precipitated by normal mouse serum. In addition broad-specificity antisera raised in mice against hamster spleen cells specifically precipitated the 45,000 and 12,000 mol. wt polypeptides (results not shown), in agreement with previous results [20]. Therefore OX18 and rabbit anti-human-β_2-microglobulin cross-react with the hamster class I heavy chain and β_2-microglobulin proteins respectively and were used for surface labelling of H14b and HA12/7 cells (Fig. 3). Surface staining of H14b cells was evident with either OX18 (Fig. 3a) or anti-β_2-microglobulin (Fig. 3c) whereas normal mouse serum did not react with the H14b cells (Fig. 3e). Immunofluorescent staining of HE1 cells using these antibodies gave results identical to those obtained with H14b cells (not shown). When HA12/7 cells were stained with the same set of antibodies, a greatly lowered level of staining was observed (Fig. 3b,d,f), consistent with the relatively lower level of class I expression noted in the mRNA analysis.

DISCUSSION

Several previous studies on the relationship of MHC class I expression to the tumorigenicity of cells have adopted a DNA transfection approach where functional class I genes, usually under the control of strong promoter, were transferred into tumour cell lines which had previously been shown to express reduced levels of class I mRNA and protein [19, 21, 22]. In general, expression of functional class I genes resulted in a loss of metastatic potential of the transfected cell lines [19, 22]. However, in two studies transfected cell lines were identified which expressed allogeneic class I proteins at the cell surface and which were lysed by appropriate cytotoxic lymphocytes yet retained unaltered metastatic potential [21, 22], suggesting that expression of class I antigens may not be sufficient for tumour rejection.

The studies reported here have shown that in HA12/7 cells (transformed by highly oncogenic Ad12) there were relatively lowered levels of class I gene expression both in culture and in solid tumours. In the HE1 and H14b cell lines (which were as tumorigenic as HA12/7) there were relatively high steady-state levels of MHC class I mRNA both in cells growing in culture and in actively growing solid tumours. Moreover class I heavy chain proteins were both synthesized and located on the surface of transformed cells. These results suggest that expression of MHC class I antigens is not incompatible with tumour formation by adenovirus-transformed hamster cells. The relative reduction in class I expression consistently seen in Ad12 transformed cells in this study and others [5, 7] may therefore accompany tumorigenesis while not necessarily being its primary cause.

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