Table 2 Correlations of anomalous components

Sample	C (%)	N (p.p.m.)	C_{heavy} (p.p.m.)	N_{light} (p.p.m.)	Xe^{136}_{CCF}	Xe^{130}_{s}	Ne-E(L)
					($\times 10^{-10}$ cc STP g^{-1})		
Murchison 2C10 f	65.0	5,190	4,500	3,800	420	1.90	≤13
Murchison 2C10 m	3.46	293	1,300	<23	0.73	0.66	16
Ratio f/m	19	18	3.5	>170	570	2.9	≤0.8
Allende B1B	49.4	>904	—	>780	860	—	—
Allende BB	79.3	>906	—	>310	180	—	—
Ratio B1B/BB	—	—	—	2.5	4.7	—	—

reliable isotopic measurements could be obtained on sub-nanogram amounts of gas.

Light nitrogen

Evidently chemical treatments (Table 1) have succeeded in enriching a distinctive, light nitrogen component in the most processed samples. The lowest $\delta^{15}N$ value of −326‰ approaches the −500‰ postulated to exist in Allende[5] and lunar surface materials[22], and called 'light planetary nitrogen' by Geiss and Bochsler[22]. Such extreme compositions cannot be achieved by means of isotopic fractionation under plausible solar system conditions, and apparently require thermonuclear reactions in stars. There is evidence for isotopically light nitrogen in lunar regolith materials which some authors attribute to a secular variation in the solar wind[24,25]. However, since no universally accepted mechanism is able to account for such changes in the Sun without other elements being affected, the subject remains controversial[22]. In principle, ion–molecule reactions in cold interstellar clouds could produce large isotopic fractionations[26] of nitrogen, but these would be of the wrong sign and affect only small molecules. Large molecules made by polymerization of small molecules inherit the same anomalies and so ion-molecule reactions are a distinct possibility for producing unusual compositions in organic polymeric material. We must stress, however, the acid and oxidation residues dealt with here are elemental, not organic carbon, and hence can be made from organic precursors only by charring. Conditions for charring (~600 K and absence of H_2, H_2O and so on) are not met in any astrophysical environment we know of, except small bodies in the solar nebula. Nitrogen, unlike Xe, is a major consituent of Allende carbon (atom fraction 10^{-10} compared with 10^{-2}) and hence must be chemically bound somehow rather than being trapped at rare, highly retentive sites[27].

Isotopically light nitrogen is rather common in the Universe, as ^{14}N forms copiously in the CNO cycle, whereas ^{15}N is produced only in rarer events. For further clues to the origin of light nitrogen let us compare the amounts of this species and other anomalous components in the various samples. We do not know whether even the most extreme $\delta^{15}N$ value of −326‰ in Allende CC actually represents the pure component, in view of the limited resolution of the analytical technique and the presence of heavier nitrogen in this and all other samples. However, for quantitative comparisons, we can calculate nominal amounts of light nitrogen in our samples by assuming their nitrogen is a binary mixture of the two most extreme compositions seen in the acid resistant residues −326‰ and +49‰ (Table 2). The calculated amount of light nitrogen (N_{light}, Table 2) in Murchison 2C10m is only an upper limit, as there is no clear evidence for light nitrogen in this sample. Both N_{light} concentrations for Allende B1B and BB are lower limits, as the data used for the calculation were obtained by the combustion technique.

The best clue to the other anomalies associated with light nitrogen is the concentration ratio for the two Murchison samples (Table 2). The finer fraction is greatly (>170×) enriched in light nitrogen and of the anomalous noble gas components only $^{136}Xe_{CCF}$ shows a similar enrichment (570×). The other two components—s-Xe and Ne-E(L)—are enriched by much smaller factors (3–4×) and are therefore thought[3,16] to be related to heavy C which is similarly enriched (3.5×).

The carbon component associated with light nitrogen and CCF-Xe is distinctive but commonplace, with $\delta^{13}C$ reaching only −32 to −38‰ in Allende B1B[3]. Again the pure component may be somewhat lighter, as small amounts of the other types of carbon were present that may not have been fully resolved by the stepped combustion technique.

Nucleosynthetic origin of CCF xenon

The origin of CCF-Xe has remained unsettled for more than a decade with opinions divided between fission of an extinct super-heavy element[28–31] and nucleosynthesis in a supernova[32,33]. No definitive test is available so far, but the association of CCF-Xe with $\delta^{15}N = -326$‰ just demonstrated argues strongly for a nucleosynthetic origin, if indeed N and CCF-Xe belong to the same phase. A second crucial experiment[34], which was performed simultaneously with our nitrogen isotope investigation and also rules out *in situ* fission, is the demonstration that fission-produced Ba, Nd and Sm are absent from samples enriched in CCF-Xe. Nonetheless, many of the objections to a nucleosynthetic origin remain[35,36]. For example, the conditions required to explain the Xe anomalies (explosive nucleosynthesis at $T = 2.0$ to 2.5×10^9 K) are incompatible with the survival of associated lighter elements (He, Ne, Ar, C) of mainly normal isotopic composition. Perhaps several sources and more than one carrier phase will have to be invoked.

Other types of nitrogen

The most highly processed samples, Allende CC and Murchison 2C10f, both show a trend towards isotopically heavier nitrogen at higher temperatures after the main release of light nitrogen (Figs 2, 4). The $\delta^{15}N$ values are unexceptional, being only +34‰ and −14‰. In view of the large temperature steps and hence low resolution of the technique, there is still a chance that a small heavy nitrogen component exists but is diluted by a residual fraction of more abundant light nitrogen. Heavy nitrogen could be expected to be associated with heavy carbon, which also evolves at high temperatures[3]. Against this argument, it is significant that heavy carbon from Murchison 2C10f (4,500 p.p.m.) appears only in the last ~1% of the release whereas the postulated heavy nitrogen (930 p.p.m., without allowance for the incomplete yield in combustion) constitutes the final 18% of the gas evolved. If we accept this result at face value, the phase containing heavy nitrogen and carbon has an exceedingly high N/C ratio of 0.21. Species of such high N/C ratios and elevated combustion temperatures are unlikely. Consequently we are inclined to attribute most of the nitrogen in the final 18% to residual light N. Assuming that heavy C has an N/C ratio of 0.008, like bulk Murchison, 2C10f and 2C10m (Table 2), then the associated nitrogen could have a $\delta^{15}N$ as high as +5,900‰. Of course, this value is only illustrative as the assumptions of only two components and a fixed N/C ratio are debatable. But it is interesting that bulk Murchison has rising $\delta^{15}N$ to +153‰ at high temperatures (Fig. 3*a*).

A demonstrable link between ^{15}N and ^{13}C would help to narrow the range of astronomical sources. It is known that substantial amounts of ^{15}N and ^{13}C are produced in novae, which are possible sources of Ne-E[37–39]. On the other hand,

normal red giant stars, while similarly enriched in ^{13}C, are depleted rather than enriched in ^{15}N. An association between heavy nitrogen and Ne-E(L) would strengthen the case for a nova origin for the progenitor (^{22}Na) of the neon gas as already argued by Arnould and Norgaard[39]. Present resolution clearly is not adequate to resolve the nitrogen component associated with s-process Xe. The nitrogen ought to be isotopically light, as expected for a red giant, and so may in part off-set the heavy nitrogen associated with Ne-E.

We thank NASA (NGL-14-001-010) (Chicago) and SERC (Cambridge) for financial support, L. Alaerts for the separation of Murchison, and members of the P.S.U. for helpful discussions.

Received 30 June; accepted 18 August 1983.

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Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells

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Rat cells transformed by the highly oncogenic adenovirus 12 lack at least two cellular proteins which are present in cells transformed by the non-oncogenic adenovirus 5 and in untransformed cells. One protein has been identified as the heavy chain of the rat class I major histocompatibility complex. This finding may explain the difference in oncogenicity between adenoviral species.

HUMAN adenoviruses are double-stranded DNA viruses some of which are capable of inducing neoplasms in rodents[1]. An interesting aspect of adenoviruses is that their oncogenic potential varies: for example adenovirus 12 (Ad12) is strongly oncogenic and induces tumours at high frequency with a short latency period (1–3 months), while adenovirus 5 (Ad5) is non-oncogenic. Virus or isolated DNA of all serotypes, however, can transform rodent cells *in vitro*[1–3]. The tumorigenicity of the resulting transformed cells in nude mice and syngeneic rats reflects the oncogenic potential of the adenovirus species used for transformation, Ad12-transformed cells being more oncogenic than Ad5-transformed cells[4–7]. The transforming activity of the DNA has been assigned to the left-most 11% (early region I, *E1*) of the viral genome[6,8,9]. This region consists of two transcriptional units, *E1a* and *E1b*[10,11], coding for proteins (T antigens) of molecular weights (MWs) 30–40,000, and 19,000 and 55,000 respectively. The transforming regions of the oncogenic and non-oncogenic species are highly homologous with respect to structural organization and nucleotide sequence[12–14], so the substantial difference in oncogenic potential between serotypes must be due to minor, but decisive, differences between the T antigens coded for by region *E1*.

How the divergence in the viral T antigens affects the oncogenic potential of the adenovirus-transformed cells is unknown. Two major concepts have been put forward: one is that the oncogenic cell might be less immunogenic to the host than its non-oncogenic counterpart; and the other that the oncogenic cell might be resistant to the cellular immune defence of the host, while the non-oncogenic cell is susceptible to it (see ref. 15 for discussion). Differences in oncogenicity between transformed cells may, therefore, be determined by the way the transformed cell interacts with the immune system of the host. This interaction might be mediated by the viral T antigens or by cellular proteins which are modulated by viral antigens. The modulation can be induction or suppression of proteins resulting in either rejection or acceptance of the non-oncogenic or oncogenic cells respectively. Such a modulation has recently been demonstrated at the level of mRNA transcription in cells transformed by the DNA tumour virus simian virus 40 (ref. 16).

We have now investigated the role of cellular proteins in oncogenicity of adenovirus-transformed cells, using an immunological approach. Highly oncogenic adenovirus-transformed baby rat kidney (BRK) cells and non-transformed BRK cells were compared immunologically by raising antibodies against them in heterologous BALB/c mice. These sera were compared with respect to their reactivity with panels of oncogenic and non-oncogenic transformed cells. We show here that oncogenicity of the transformed cells is specifically correlated with the absence of 32,000- and 45,000-MW cellular proteins (32K and 45K respectively). The latter protein is identified as the heavy chain of the rat class I major histocompatibility complex (MHC).

0028-0836/83/430771—05$01.00

Suppression of nonviral protein expression

BALB/c mice were immunized with either nontransformed primary kidney cells of 6-day-old Wag-Rij rats or highly oncogenic cells transformed by a plasmid containing the left-terminal *Eco*RI-C fragment of Ad12 DNA (pAd12RIC), which comprises the entire early region 1[13,17,18]. Sera from several mice were used for immunoprecipitation of ^{35}S-methionine-labelled extracts of primary cultures of BRK cells and pAd12RIC-transformed cells. Sera raised against primary rat kidney cells in seven different mice precipitated only a limited number of proteins (Fig. 1, lanes 2–8). A large amount of a 38,000-MW (38K) protein was precipitated from the lysate of primary cells (lanes 2*a*, 4*a*, 8*a*) and was absent in the transformed cell lysate with at least three individual antisera (*b* lanes). Close inspection of the original X-ray film also revealed a 32K protein in untransformed cells which was absent in the transformed cells. When sera raised in BALB/c mice against Ad12-transformed cells were used for immunoprecipitation, no major differences could be observed between proteins precipitated from untransformed and transformed cells (results not shown).

To investigate further which viral genes are involved in the regulation of the 32K and 38K proteins in the transformed cells, a pooled mouse serum was used for an analysis of a panel of adenovirus-transformed cell lines. The 32K protein is also present in a cell line transformed by a plasmid containing region *E1* of Ad5 DNA (pAd5*Xho*IC; Fig. 2), which suggests that Ad12 sequences suppress the expression of the 32K protein after transformation of the rat cells whereas Ad5 sequences do not. Rat cells transformed by Ad12 region *E1* plasmids in which either the 19K (ref. 19) or the 54K (ref. 6) *E1b* proteins are truncated due to frameshift mutations also lack the 32K protein (Fig. 2, lanes 3, 6). This indicates that the suppression of the 32K protein is not a function of the Ad12 *E1b* proteins but, more likely, an effect of the Ad12 *E1a* region. Thus Ad5 region *E1a* would lack this property.

It could be argued, however, that Ad5 region *E1a*, like Ad12 *E1a*, switches off the expression of the 32K protein, but that the Ad5 *E1b* proteins antagonize this effect. To exclude this possibility, Ad5-transformed cells lacking either the 21K or 55K *E1b* proteins, or both, were investigated. All three transformed cell lines produced the 32K protein (Fig. 2, lanes 7, 4 and 5 respectively), indicating that the Ad5 *E1b* proteins are not involved in the regulation of the expression of 32K. This is further supported by experiments with cell lines transformed by hybrid plasmids[17] consisting of Ad5 *E1a* and Ad12 *E1b* (pAd512), and vice versa (pAd125). These experiments also show (Fig. 2, lanes 8, 9) that the absence of the 32K protein is correlated with the presence of Ad12 region *E1a*, even when the *E1b* sequences originate from Ad5.

As discussed in the accompanying paper[18], cells containing Ad12 region *E1a* are oncogenic in immunocompetent rats, while cells containing Ad5 *E1a* are not. Since only cells expressing Ad12 *E1a* sequences lack the 32K protein, the 32K protein may be involved in the rejection of non-oncogenic adenovirus-transformed cells.

There is no direct correlation between the occurrence of the 38K protein and the presence of Ad5 *E1a* sequences (Fig. 2).

38K protein and β_2-microglobulin

To investigate whether the 32K protein is a membrane protein, cells were labelled with radioactive iodine and extracts of the cells were immunoprecipitated with the pooled BALB/c anti-BRK serum used in the previous experiment. No 32K protein could be precipitated from primary and transformed cells (Fig. 3, lanes 1*b*, 2*b*), which suggests that the protein is probably not a membrane protein. Very prominent 38K and 12K proteins, however, were precipitated from primary cells, but not from Ad12-transformed cells. Since several membrane proteins of 40–45,000 MW and coded for by the class I MHC are known to be associated with a small 12K molecule, β_2-microglobulin[20–22], we used the same ^{125}I-labelled cell extract for

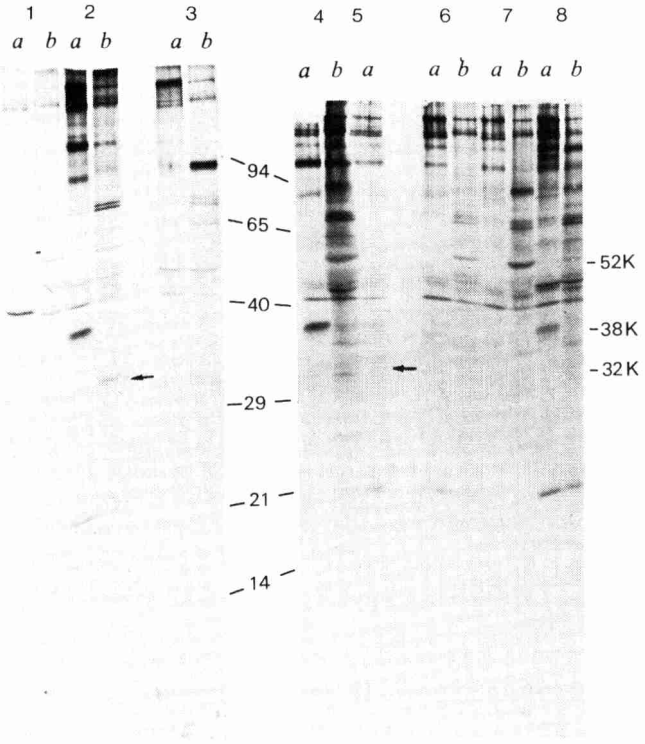


Fig. 1 SDS–polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of ^{35}S-methionine-labelled cells with antisera raised against primary BRK cells. Antisera were prepared by immunization of 10-week-old BALB/c mice with 10^7 cells of cultures of primary BRK cells derived from 6-day-old Wag-Rij rats (a highly inbred Wistar strain obtained from the Radiobiological Institute, Rijswijk). Three intraperitoneal injections were given at 10-day intervals; the first injection was combined with complete Freund's adjuvant. The mice were bled 14 days after the last injection. Cell-labelling and subsequent immunoprecipitation procedures have been described previously[17]. Antisera from seven different mice (lanes 2–8) were used for immunoprecipitation of extracts of primary BRK cells (lanes *a*) or extracts of cells transformed by a plasmid containing Ad12 early region *E1* (pAd12RIC)[13] (lanes *b*). The molecular weight of several marker proteins are indicated. Lane 1 represents a control precipitation with normal mouse serum.

immunoprecipitation with a commercial antiserum against human β_2-microglobulin, which cross-reacts with rat β_2-microglobulin chains (our unpublished results). A considerable amount of β_2-microglobulin was precipitated from primary BRK cells (Fig. 3, lane 3*d*) and Ad5-transformed cells (lane 4*d*) but the amount precipitated from Ad12-transformed cells was substantially reduced (lane 5*d*). No heavy-chain class I MHC molecules were co-precipitated with β_2-microglobulin. This might be explained by the fact that the antiserum only reacts with free β_2-microglobulin molecules because it only recognizes the evolutionarily conserved domain which may be shielded when the molecule is complexed to the heavy chain. These free chains may have been dissociated from the MHC heavy chain as an artefact of the immunoprecipitation procedure after extraction of the complex from the membrane. The strong reduction of the amount of β_2-microglobulin on the membrane of Ad12-transformed cells suggests also that the expansion of class I MHC molecules on the surface of these cells might be suppressed.

Class I MHC molecule expression

The presence of class I MHC antigens on the cell surface of adenovirus-transformed rat cells was further investigated using specific rat alloantisera. The rat MHC, the *RT1* complex, comprises five loci coding for histocompatibility antigens. Two loci, *RT1.A* and *RT1.E*, control the expression of the classical serologically detectable class I antigens[23]. All primary and trans-

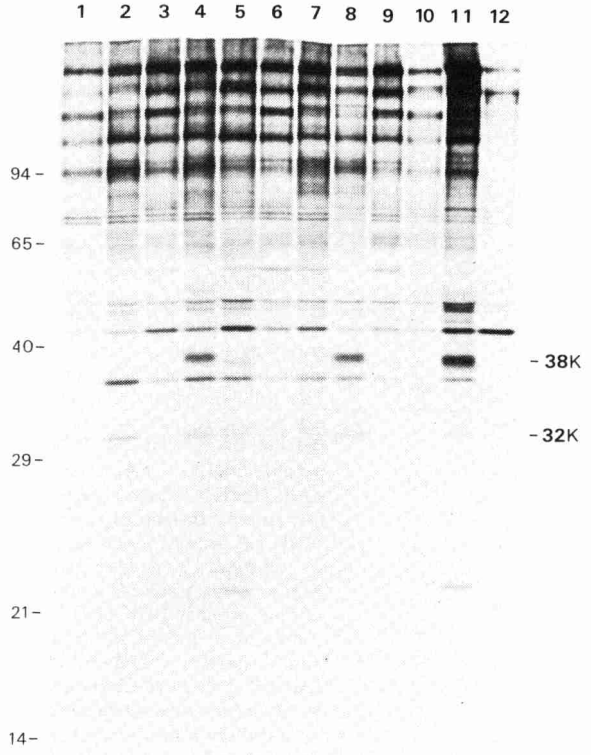


Fig. 2 SDS–polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of ^{35}S-methionine-labelled adenovirus-transformed cell lines with mouse antiserum raised against primary BRK cells. The antisera for lanes 2, 4, 5 and 8 (see Fig. 1 legend) were pooled and used for immunoprecipitation of rat cell lines transformed by the following plasmids (see Table 1 for expression of viral genes in the transformed cells): lane 1, pAd12RIC; 2, pAd5*Xho*C; 3, pAd12*Hin*dIIIG; 4, pAd5*Hin*dIIIG; 5, pAd5*Hpa*IE; 6, pAd12dl*Acc*; 7, pAd5dl*Sac*; 8, pAd512; 9, pAd125; 10, primary BRK cells; 11, primary BRK cells precipitated with a normal mouse serum.

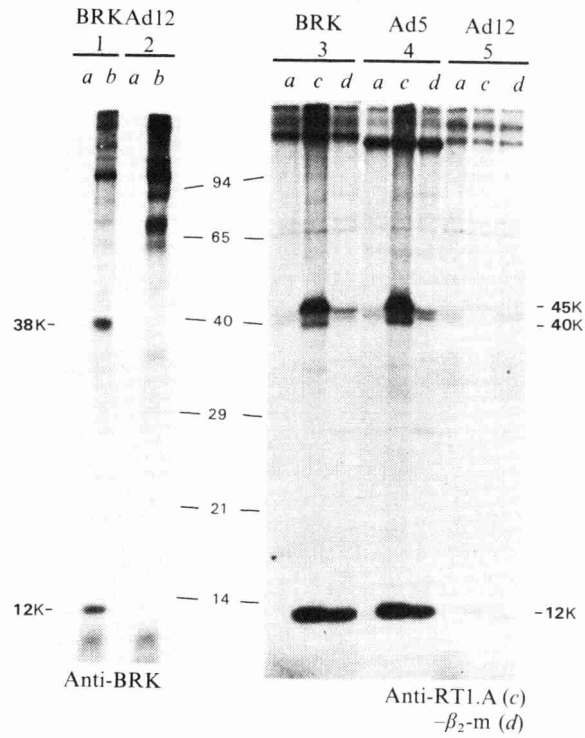


Fig. 3 SDS–polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of ^{125}I-labelled cells with mouse anti-primary BRK antiserum, an anti-Wag-Rij alloantiserum and a heteroantiserum against β_2-microglobulin. Monolayer cultures of the cells were washed with phosphate-buffered saline (PBS) and scraped off the plates with a rubber policeman. 10^7 cells in 1 ml of PBS were transferred to an Iodogen-coated Pyrex glass tube and 1 mCi of Na^{125}I was added[33]. After incubation at room temperature for 15 min, the cells were washed with PBS and extracted with immunoprecipitation buffer[17]. Extracts of the following cells were used for immunoprecipitation: lanes 1, 3, primary BRK cells; 2, 5, pAd12RIC-transformed BRK cells; 4, pAd5*Xho*C-transformed BRK cells. The antisera used were a normal mouse antiserum (lanes *a*), the mouse anti-primary BRK antiserum (see Fig. 2 legend), a Lewis anti-Wag-Rij hyperimmune rat alloantiserum (lanes *c*) and an anti-human β_2-microglobulin antiserum (anti-β_2-m; Dako; lanes *d*).

formed rat kidney cells described in this study originate from Wag-Rij rats, a highly inbred Wistar strain, carrying the *RT1*^{u} haplotype. Hyperimmune sera against this haplotype raised in Brown Norway (*RT1*^{n}) or Lewis (*RT1*^{l}) rats precipitated almost exclusively 45K and 12K proteins from ^{125}I-labelled primary BRK cells (Fig. 3, lane 3*c*), representing the *RT1.A* heavy chain and β_2-microglobulin respectively (compare with lane 3*d*). A similar amount of *RT1.A* was found in cells transformed by Ad5 *E1* DNA (Fig. 3, lane 4*c*). However, cells transformed by Ad12 *E1* DNA contained a greatly reduced amount of the 45K protein (Fig. 3, lane 5*c*). Similar results were obtained when cells were metabolically labelled with ^{35}S-methionine and subsequently precipitated with the Lewis anti-Wag-Rij serum (Fig. 4*A*, lanes 1*b*, 3*b*, 8*b*).

Suppression of *RT1.A* by Ad12 *E1a*

To investigate which viral gene determines the suppression of *RT1.A*, a panel of transformed cells was analysed by immunoprecipitation with the anti-*RT1.A*^{u} antiserum. Only cells containing the Ad12 *E1a* region had strongly reduced amounts of *RT1.A* (Fig. 4). All cells containing the *E1a* region of Ad5, including those harbouring Ad12 *E1b* sequences, expressed normal levels of *RT1.A* (Fig. 4A, lanes 1–3, 7). Interestingly, suppression of *RT1.A* parallels that of the 32K protein; thus oncogenicity of adenovirus-transformed cells in syngneic immunocompetent rats is strictly correlated with the suppression of the class I MHC antigens and of the 32K protein. These effects are specifically induced by Ad12 *E1a*, but not Ad5 *E1a*, region.

The almost complete absence of alloreactive MHC class I molecules on the surface of Ad12-transformed cells may have several causes. To test whether the phenomenon is due to the lack of β_2-microglobulin as is the case in Daudi cells[24], we investigated metabolically labelled cells for the presence of β_2-microglobulin. From ^{35}S-methionine-labelled Ad12-transformed cells, which lack the *RT1.A* heavy chain completely, a normal amount of β_2-microglobulin can be precipitated (Fig. 4*B*, lane 2*b*), as compared with Ad5-transformed cells (Fig. 4*B*, lane 2*a*). Thus, the absence of *RT1.A* on the membrane of Ad12-transformed cells is not due to a lack of β_2-microglobulin synthesis. A second possibility was that the absence of MHC class I molecules on the cell surface can be explained by the appearance of 'alien', 'extra' or 'inappropriate' MHC specificities on the cell surface as has been reported elsewhere[25,26]. The mechanism of these phenotypic switches is not known. Furthermore, the loss, or alteration, of allogeneic reactivity on tumour cells may be the result of structural alterations of the class I molecule due to association with products of viral replication or expression[27,28].

To exclude these possibilities the expression of MHC class I-specific mRNA from several adenovirus-transformed cell lines was examined using a cloned DNA probe[29] containing sequences of the human *HLA B7* gene (Fig. 5). Since the probe contains sequences of the constant (third) domain, it should react with

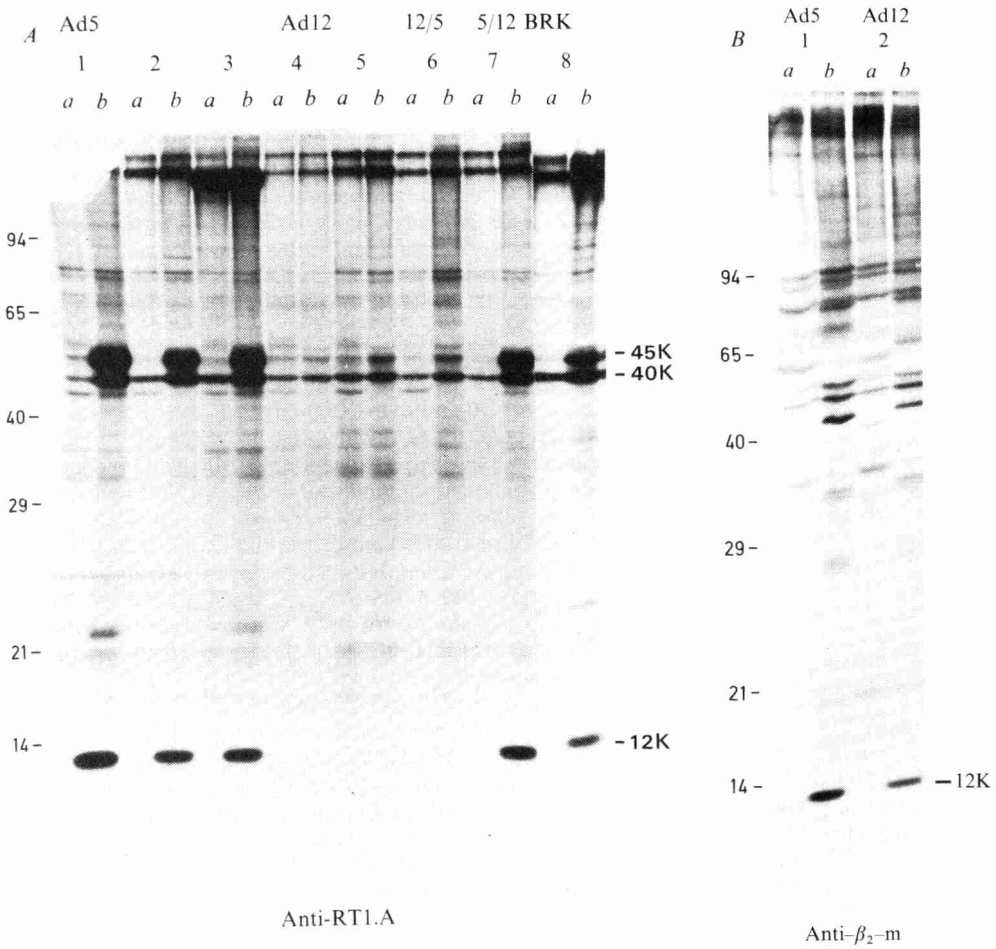
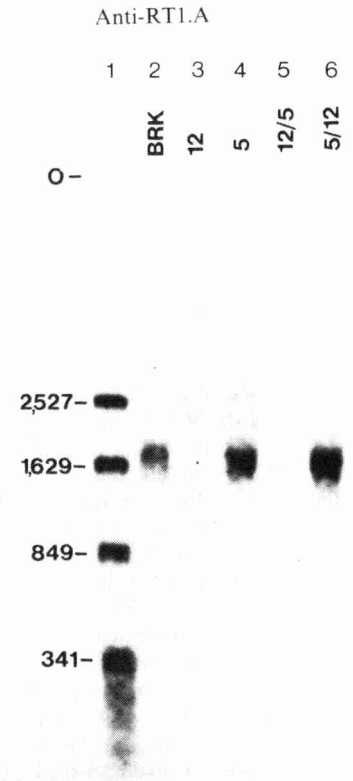


Fig. 4 SDS–polyacrylamide gel electrophoresis of proteins immunoprecipitated from extracts of ^{35}S-methionine-labelled cells with an anti-Wag-Rij alloantiserum (*A*) and an anti-β_2-microglobulin serum (*B*). The following plasmid-transformed cell lines were used (see Table 1 for details): pAd5*Xho*C (*A*, *B*, lanes 1); pAd5*Hin*dIIIG (*A*, lane 2); pAd5*Hpa*IE (*A*, lane 3); pAd12RIC (*A*, lane 4; *B*, lane 2); pAd12*Hin*dIIIG (*A*, lane 5); pAd125 (*A*, lane 6) and pAd512 (*A*, lane 7). *A*, lane 8, represents a control consisting of normal primary BRK cells. Lanes *a* represent a normal rat serum (*A*) and a normal rabbit serum (*B*). Lanes *b* represent a Lewis anti-Wag-Rij hyperimmune alloantiserum (*A*) or a rabbit anti-human β_2-microglobulin antiserum (*B*).



Anti-β_2-m

Fig. 5 Class I MHC transcripts in primary and adenovirus-transformed BRK cells. Cytoplasmic RNA was isolated by resuspending a cell pellet in 10 mM Tris-HCl (*p*H 7.8), 1 mM EDTA, 150 mM NaCl, 0.65% NP40 at 4 °C. The nuclear pellet was removed, the supernatant extracted with phenol–chloroform and the RNA precipitated in ethanol. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography[34]. One μg of poly(A)$^+$ RNA (selected once) was separated by electrophoresis in 1.0% agarose–formaldehyde gels[35] for 4 h at 7.5 V cm^{-1} and thereafter blotted overnight onto a nitrocellulose filter in a buffer consisting of 16×SSC, 2.2 M formaldehyde. The blot was baked for 2 h at 80 °C and prehybridized for at least 3 h at 42 °C in a buffer containing 0.75 M NaCl, 50 mM sodium phosphate (*p*H 7.4), 5 mM EDTA, 0.1% SDS, 5×Denhardt solution (0.1% each of Ficoll, bovine serum albumin and polyvinylpyrrolidone), 0.25 mg denatured calf thymus DNA per ml and 50% formamide. Subsequently, the blot was hybridized for 16 h at 42 °C in the same buffer, containing 5×10^6 c.p.m. ml^{-1} of a nick-translated human *HLA B7* cDNA probe[29], washed three times in 2×SSC, 0.1% SDS at 50 °C for about 2 h and autoradiographed for 40 h. Rat cell lines transformed by the following plasmids were used: lane 2, primary BRK cells; 3, pAd12RIC; 4, pAd5*Xho*C; 5, pAd125; 6, pAd512. Lane 1 represents a 5′-end-labelled *Hae*III digest of phage M13 mp8 DNA (molecular weights are given in base pairs). O, origin.

any MHC class I heavy-chain mRNA present in rat cells. Figure 5, lane 2, shows that the probe hybridizes to a 1.7-kilobase (kb) mRNA present in RNA isolated from normal BRK cells. This band probably represents the RNA coding for the *RT1.A* heavy chain. The amount of MHC class I-specific mRNA is greatly reduced in transformed cells harbouring Ad12 *E1a* sequences, irrespective of the identity of the *E1b* region (Fig. 5, lanes 3, 5). In contrast, transformed cells containing the Ad5 *E1a* region do express normal amounts of *RT1.A* mRNA, again independent of the identity of the *E1b* region (lanes 4, 6). These results clearly demonstrate that the absence of *RT1.A* antigenic determinants in Ad12-transformed cells is due to a mechanism regulating the mRNA production, rather than modifying the antigenic properties of the *RT1.A* molecules.

Table 1 Proteins present in a number of adenovirus-transformed rat cell lines with different oncogenic potential

Adenovirus-transformed rat cell line	Expression of Ad5 or Ad12 region		Protein present in cellular extract		
	E1a	*E1b*	32K	*RT1.A*^{a} (45K)	β_2-microglobulin (12K)
BRK (untransformed)	—	—	+	+	+
pAd5*Xho*IC[17]	5	5	+	+	+
pAd5*Hin*dIIIG[19]	5	5(19K)	+	+	+
pAd5dl*Sac*[19]	5	5(58K)	+	+	+
pAd5*Hpa*IE[17]	5	—	+	+	+
pAd12RIC[13]	12	12	−	−	+
pAd12*Hin*dIIIG[6]	12	12(19K)	−	−	+
pAd12dl*Acc*[19]	12	12(54K)	−	−	+
pAd512[17]	5	12	+	+	+
pAd125[17]	12	5	−	−	+

+, Present; −, absent or strongly reduced.

Discussion

In an attempt to understand why Ad12-transformed cells are capable of escaping the immune defence of immunocompetent rats, we investigated possible differences in the proteins contained in various oncogenic and non-oncogenic adenovirus-transformed cells (Table 1). Two proteins, normally present in untransformed and Ad5-transformed cells, were absent from Ad12-transformed cells. One of these proteins has a molecular weight of 32,000, while the other was identified as the heavy chain (45K) of the rat class I MHC *RT1.A*. The light chain, β_2-microglobulin, was present in normal amounts in Ad12-transformed cells and the suppression of the 45K protein was shown to occur at the level of mRNA synthesis. The disappearance of both the 32K and 45K proteins is strictly correlated with the presence of Ad12 region *E1a*, and is totally independent of the identity of the *E1b* region (Table 1). From tumorigenicity data, it can be concluded that oncogenicity of the transformed cells in immunocompetent rats is also strictly correlated with the expression of Ad12 *E1a* sequences[18]. Thus, the essential question is whether the reduced level of class I MHC molecules can account for the high oncogenicity of Ad12-transformed cells. Our accompanying article[18] shows that rejection of transformed cells is probably an activity of cytotoxic T cells (CTLs) from which Ad12 *E1a*-transformed cells are able to escape. CTL activity, like a number of cell–cell interactions in the immune system, is mediated via antigens encoded in the MHC, whose presence on the cell surface is therefore necessary[30,31]. Consequently, tumour cells expressing viral antigens, but not class I MHC antigens, cannot be killed through T-cell cytotoxicity[31]. This phenomenon, generally known as 'MHC restriction', can explain the resistance of the class I MHC-deficient Ad12-transformed cells against the cellular immune response of the host, resulting in growth into a tumour. Experiments showing the deficiency of a CTL response to Ad12-transformed cells *in vitro* are published in our accompanying paper[18].

The possibility cannot be excluded that Ad12 only tranforms target cells which lack expression of class I MHC genes. Experiments to test this possibility are in progress.

To our knowledge, the findings reported here represent the first demonstration of an interaction of a viral gene product (that of Ad12 *E1a*) with the control of a specific cellular gene which appears to influence the oncogenic potential of the cells. Our conclusions are confirmed by a recent report[32] demonstrating that SV40-transformed mouse C3H fibroblasts, which were selected for high oncogenic potential in syngeneic mice by serial passage, had lost expression of the *H–2K*^{k} allele of the murine class I MHC. These highly oncogenic cells were resistant to killing by secondary anti-SV40 CTLs, in contrast to non-oncogenic cells which were lysed, unless the reaction was blocked with anti-*H–2K*^{k} antibodies. Taking all the data together, we conclude that the MHC class I-restricted CTL response may represent an important mechanism for *in vivo* rejection of DNA tumour virus-transformed cells, and that oncogenicity may result from suppression of class I major histocompatibility antigens.

We thank Drs L. C. Paul and L. Vaessen for generous gifts of rat alloantisera and Dr S. M. Weissman for the cloned cDNA probe coding for HLA B7. The work was supported in part by the Dutch Foundation for Pure Research (ZWO) through the Foundation for Fundamental Medical Research (FUNGO).

Received 16 June; accepted 2 September 1983.

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