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Coxsackie and adenovirus receptor (CAR)-dependent and major histocompatibility complex (MHC) class I-independent uptake of recombinant adenoviruses into human tumour cells

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The role of two receptors, previously proposed to mediate the entry of adenoviruses into human cells, the coxsackie and adenovirus receptor (CAR) and the major histocompatibility complex (MHC) class I heavy chain has been investigated. The expression of MHC class I in many tumours is reduced or absent, therefore if this were a means by which adenoviruses gained entry into cells, it would have important implications for their application in cancer treatment. In order to determine if MHC class I heavy chain is involved in adenovirus type 5 (Ad5) uptake, the binding of recombinant Ad5 fibre knob domain (which mediates viral attachment) to human cell lines that had greatly different levels of surface MHC class I was studied. We also created derivatives of a non-permissive Chinese hamster ovary (CHO) cell line that expressed human class I (HLA-A2) and found that these cells did not bind fibre or take up virus. In addition, the extracellular domain of CAR was expressed in E. coli and used to generate a polyclonal anti-CAR antibody. This antibody blocked both ^{125}I labelled fibre knob binding and virus uptake. Thus CAR, and not MHC class I, is a receptor for human adenoviruses in cultured tumour cells. Tissue CAR levels may therefore be an important factor in the efficiency of adenovirus-mediated gene therapy.

Keywords: *CAR; MHC class I; adenovirus; viral receptor; viral uptake*

Introduction

Recombinant human adenoviruses are potentially useful gene therapy vectors because of their ability to mediate the transfer of therapeutic genes to a wide variety of cells.[1] While this broad host range is an advantage, the lack of cell specificity may also be detrimental if adenoviruses encode proteins with potential cytotoxic effects.[2] In addition, the *in vivo* efficiency of gene delivery may be reduced by attachment of the virus to cells that are not actively targeted. Hence, the targeting of therapeutic genes to specific cells is an important issue in the application of recombinant adenoviruses for gene therapy.

Human adenoviruses enter susceptible cells by receptor-mediated endocytosis, initiated by the interaction of the fibre protein with a cell surface receptor.[3,4] The adenovirus 5 (Ad5) fibre is a homotrimer, where each monomer consists of three domains: an amino-terminal tail which associates with the penton base protein, a shaft consisting of a 15 amino acid sequence repeated 22 times and a carboxy-terminal globular knob domain, which interacts with a host cell surface receptor.[4] The fibre–receptor interaction is accompanied by another binding event in which the penton base, via an exposed RGD motif, interacts with $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins on the cell surface, leading to internalisation of the virus.[5] Two proteins have been proposed to be the receptor for the fibre of subgroup C adenoviruses, the coxsackie and adenovirus receptor (CAR) and MHC class I heavy chain.[6,7] CAR is a 46 kDa transmembrane glycoprotein with an extracellular domain containing two immunoglobulin-like folds. The presence of CAR is essential for fibre-dependent uptake of adenoviruses and expression of the CAR protein has recently been used to facilitate the entry of Ad5 into a number of cell types which are not generally susceptible to the virus.[8–10] MHC class I molecules are cell-surface glycoproteins consisting of a polymorphic heavy chain non-covalently linked to an invariant chain, beta-2-microglobulin (β_2m). The α_2 domain of the heavy chain has been proposed to participate in the binding of Ad5 fibre.[11] The involvement of MHC class I molecules in adenovirus attachment to susceptible cells remains, however, unclear.

The aim of this study was to assess the relative contribution of each proposed receptor to the uptake of adenovirus 5, by investigating the binding of recombinant fibre knob and uptake of Ad5 by a variety of cell lines that differed in their expression of CAR and MHC class I molecules.

Results

Relationship between MHC class I surface expression, fibre knob binding and viral uptake

Tumour cell lines were screened for the expression of MHC class I and for their ability to bind fibre knob. The

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W6/32 monoclonal antibody recognises a monomorphic determinant on class I heavy chains that are associated with β_2m. This antibody was used to detect the cell surface expression of MHC class I on each cell line (Figure 1). The displacement of the peak to the right compared with the control signifies the expression of MHC class I molecules on the surface of cervical adenocarcinoma HeLa and the small cell lung carcinoma A549 cells. In contrast, the colorectal tumour cell lines HCT8 and HRT18 do not express surface class I molecules. Fen 2 and Fen 3 are closely related bladder carcinoma cell lines. Fen 2 cells are class I-negative, their stable transfection with β_2m generated Fen 3 cells with restored surface MHC class I expression[12] (Figure 1).

In order to study fibre binding, recombinant fibre knob was labelled with ^{125}I and shown to be trimeric by analysis of boiled and native samples by SDS/PAGE and FPLC (Ref. 13 and data not shown). Fibre knob binding was quantified by measuring the amount of ^{125}I bound after incubation of cell monolayers with increasing amounts of labelled protein. To ensure that the protein was not internalised, the cells were incubated at 4°C. Fibre knob binding reached saturation after 8 h (data not shown). The results were analysed using the ORIGIN program and fitted to a non-linear, least square model to yield apparent dissociation constants (K_d) and number of receptors per cell (Table 1). Model fitting demonstrated the presence of a single class of high affinity binding site and a test for co-operativity yielded a Hill coefficient of 1.0, demonstrating the absence of any co-operative binding of fibre knob to each cell line, irrespective of MHC class I expression. In addition, the number of receptors per cell was higher in the class I-negative Fen 2 cell line compared with class I-positive Fen 3 cells, suggesting a lack of correlation between MHC class I expression and fibre binding. In order to investigate this further, CHO cells, which did not bind fibre knob (Table 1), were transfected with an MHC class I HLA-A2 heavy chain construct (generating CHO-A2 cells) and fibre knob binding measured. No fibre receptors were detected on CHO-A2 cells.

Uptake of whole virus particles was studied by measuring expression of a reporter gene encoding a green fluorescent protein (GFP) placed under the control of a CMV promoter in a recombinant adenovirus. CHO and CHO-A2 cells were transduced with the Ad5CMVEGFP replication defective virus (with E1 and E3 genes deleted) and GFP assayed by flow cytometry (Figure 2). Very low expression of GFP in CHO-A2 cells (similar to that in control CHO cells) was detected, thus demonstrating that these cells failed to take up virus.

Relationship between CAR expression, fibre knob binding and viral uptake

Expression of CAR was studied at the protein and mRNA level. Protein was detected with a rabbit polyclonal anti-CAR antibody, generated with the extracellular domain of CAR expressed in *E. coli*. Western blotting identified a protein of approximately 46 kDa, in agreement with the size of full-length CAR, in extracts of A549, HeLa,

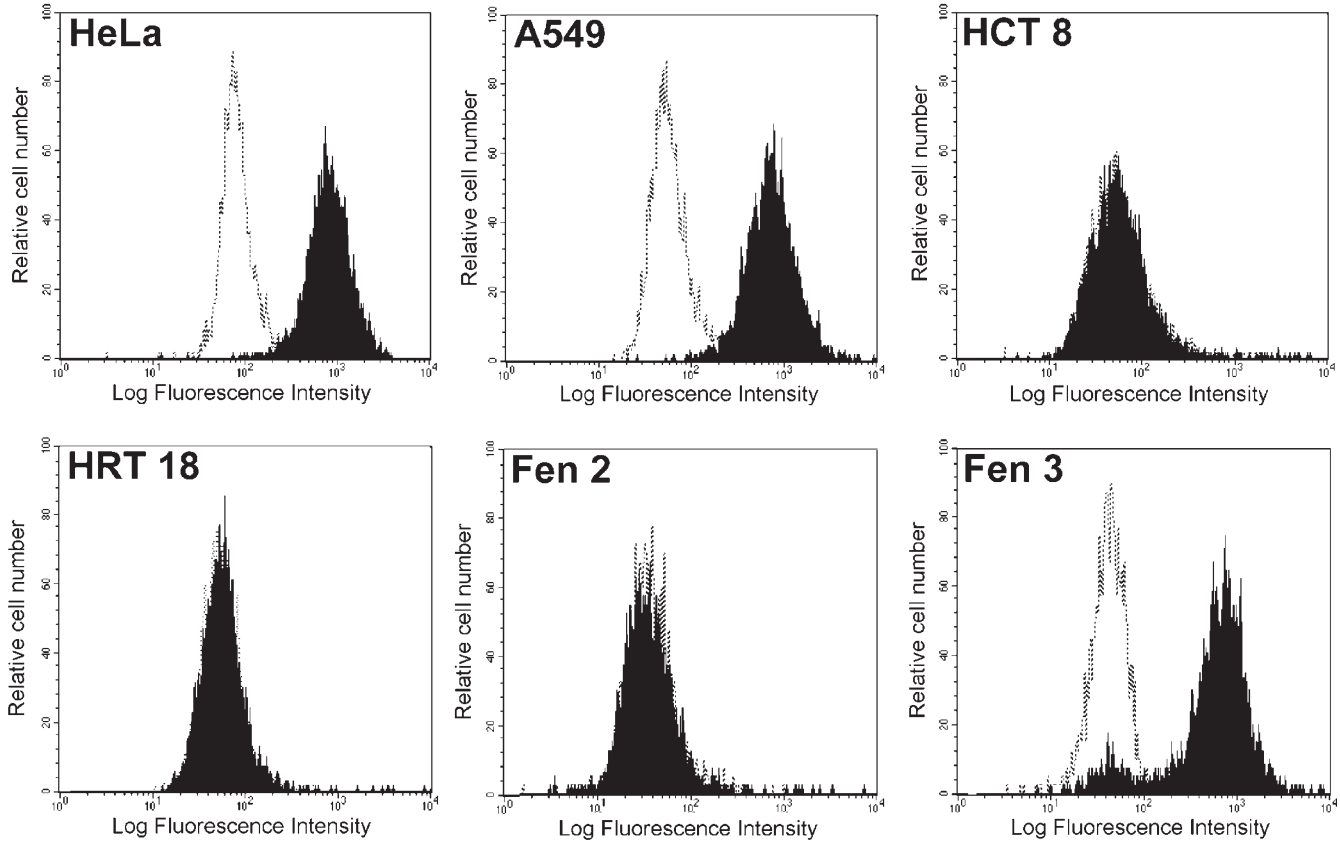

Figure 1 *Analysis of MHC class I surface expression on human tumour cell lines. Equivalent numbers of cells were analysed by flow cytometry using FITC-labelled W6/32. An isotype matched IgG was used as a control.*

Table 1 Characteristics of fibre binding, MHC class I and CAR expression in the cell lines

Cell type	*K_d (pM) ± s.e. (n = 3)*	*No. receptors/ cell ± s.e. (n = 3)*	*MHC class I expression*	*CAR expression*
A549	205 ± 25	11100 ± 2477	+	+
HeLa	209 ± 41	11500 ± 2300	+	+
HCT 8	75 ± 28	3627 ± 910	–	+
HRT 18	92 ± 20	8299 ± 1935	–	+
Fen 2	276 ± 95	17703 ± 1146	–	+
Fen 3	110 ± 18	12418 ± 1484	+	+
CHO	NSB	NSB	–	–
CHO-A2	NSB	NSB	+	–
CHO-CAR	307 ± 78	81335 ± 20765	–	+

Receptor binding was assayed as described in Materials and methods. Computerised model fitting (Origin) of the data yielded K_d values. Values shown are the mean of three independent experiments. Flow cytometry was used to determine relative levels of surface MHC class I and CAR expression on the cell lines, using the W6/32 and RmcB antibodies, respectively, as described in Materials and methods. NSB, no significant binding; + signifies expression of MHC class I or CAR (see Figures 1 and 4).

HRT18, HCT8, Fen 2 and Fen 3 cells and RT-PCR analysis confirmed the presence of CAR transcripts in those cell lines (data not shown). In contrast, the CAR protein and its corresponding RNA were not present in CHO cells. Cell surface localisation of CAR was detected by flow cytometry in all studied CAR-expressing cell lines (Figure 3). Notably, the anti-CAR antibody did not cross-react with surface MHC class I molecules on CHO-A2 cells (and anti-class I antibody did not cross-react with CAR on CHO-CAR cells). In addition, immunofluorescence microscopy using either the polyclonal anti-CAR antibody or the monoclonal anti-CAR RmcB antibody[6] showed a punctate plasma membrane localisation of CAR (Figure 4). Analysis of fibre binding to the studied cell lines showed a good correlation with surface CAR expression (Table 1). Studies on the uptake of virus particles (Ad5CMVEGFP) by CHO-CAR cells showed GFP expression in 26% of viable cells, thus confirming the ability of CAR to function as an Ad5 receptor (Figure 2).

The polyclonal anti-CAR antibody blocks ^{125}I fibre binding and viral uptake

The relative roles of CAR and MHC class I molecules in the attachment of adenovirus 5 to human cells were further studied by blocking either fibre knob binding or uptake of whole virus particles (measured by luciferase expression from the Ad5luc3 recombinant virus) with polyclonal anti-CAR immunoglobulins (Figure 5a and b). We analysed both class I-positive (A549) and class I-negative (Fen 2 and HRT18) cell lines. Anti-CAR immunoglobulins inhibited both processes in a concentration-dependent manner in all three cell lines irrespective of class I expression, while control IgG had no effect.

Discussion

The development of new gene therapy vectors will benefit from a detailed knowledge of the mechanism of adenovirus attachment and internalisation. Restriction of viral tropism to selected cells or the introduction of a new binding specificity will require modification of the interaction between fibre and its natural receptors. Two receptors have been reported to be involved in the binding of Ad5 to susceptible cells, CAR and the MHC class I heavy chain.[6,11] The dependence of adenovirus attachment on MHC class I molecules would have significant consequences for their application in cancer therapy[13,14] since most tumours fail to express these molecules[15] and this would have to be taken into account in the design of new vectors. Therefore the aim of this study was to determine the involvement of these two types of molecules in adenovirus attachment.

We showed that a soluble trimeric fibre knob of Ad5 bound with high affinity to HCT8, HRT18 and Fen 2 cells which are MHC class I negative. Moreover expression of class I molecules on Fen 3 cells (which were derived from Fen 2 by stable transfection with β-microglobulin) did not increase fibre knob binding. A very good correlation, however, was evident between fibre knob binding and cell surface expression of CAR. To study fibre–receptor interaction further, a cell system in which CAR was not involved was developed by transfection of CHO cells, which are not permissive for Ad5 infection, with the HLA-A2 heavy chain. Cell surface expression of class I molecules on CHO-A2 cells was detected by flow cytometry but no binding of fibre knob or uptake of recombinant Ad5 could be demonstrated. Furthermore, fibre knob binding and recombinant Ad5 uptake by human cells were inhibited by a polyclonal anti-CAR antibody in a concentration-dependent manner, irrespective of class I expression.

It has been reported that by reverse antibody biopanning of a phage display hexapeptide library a peptide was identified which bound to Ad5 fibre knob and to a monoclonal anti-fibre antibody that inhibited Ad5 attachment to HeLa cells.[11] This peptide was described as a mimotope of the fibre knob receptor and was shown to be homologous to the α_2 domain of the MHC class I heavy chain. The corresponding MHC class I sequence was capable of binding Ad5 and Ad2, but not Ad3, consistent with previous observations that Ad2 and Ad5 do not compete with Ad3 for receptor binding.[4] Our data, however, do not support this finding. A lack of correlation between transduction of melanoma cells with recombinant adenoviruses and expression of MHC class I and integrins has also been recently reported.[16]

Materials and methods

Cell culture

Dulbecco modified Eagle's medium (DMEM) and cell culture supplements were obtained from ICN Flow (Basingstoke, UK) and foetal calf serum (FCS) from PAA Laboratories (Consett, UK). HeLa, A549, HCT8 and HRT18 were maintained as monolayers in DMEM plus 10% FCS. CHO cell lines were maintained in MEMα (Life Technologies, Paisley, UK) supplemented with 10% foetal calf serum. Fen 2 and Fen 3 cell lines were kindly provided by A Nouri (Department of Medical Oncology, Royal London Hospital, London, UK) and CHO-CAR cells by R Finberg (Division of Infectious Disease, Dana-Farber Cancer Institute, Boston, MA, USA).

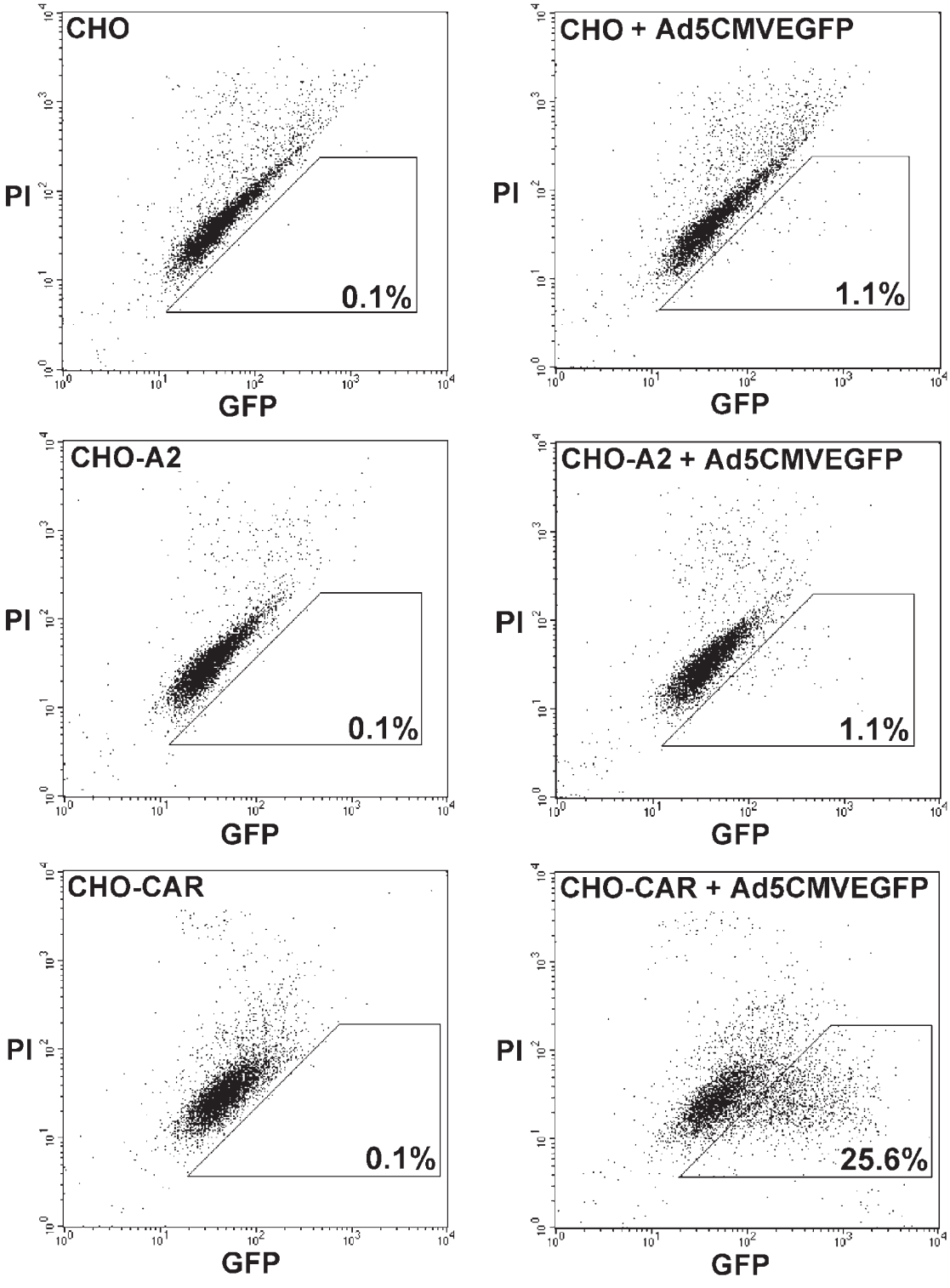

Figure 2 *Uptake of recombinant Ad5 virus by CHO, CHO-A2 and CHO-CAR cells. Cells were exposed to virus for 1 h at 4°C, washed to remove non-specifically bound virus and incubated at 37°C for 24 h. Expression of the green fluorescent protein (GFP) transgene product was assayed by flow cytometry following transduction of CHO cell lines with Ad5CMVEGFP. Viable cells were detected by propidium iodide (PI) staining.*

Isolation of stably transformed cell lines

One day before transfection CHO cells were plated in 10-cm dishes so that the monolayer would be 50% confluent on the following day. The cells were then transfected with 5 μg of A2RSV5neo[17] or with RSVneo control plasmid using Superfect transfection reagent (Qiagen, Crawley, UK), according to the manufacturer's instructions. After 24 h, the cells were grown in DMEM supplemented with 10% FCS and 1 mg/ml geneticin (Sigma, Poole, UK), which was changed after 5 days. After 10 days, individ-

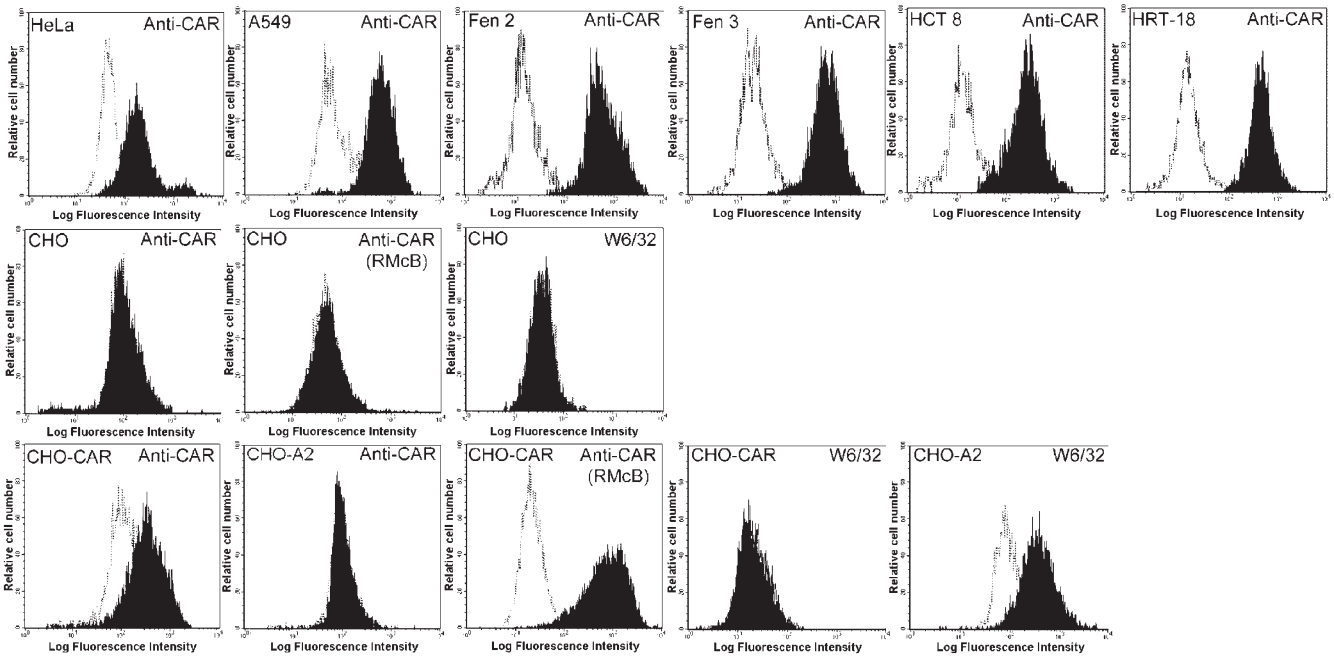

Figure 3 *Expression of surface CAR and MHC class I molecules on human tumour, CHO, CHO-A2 and CHO-CAR cell lines. Cells were analysed by flow cytometry using anti-CAR (1:10) or RmcB (1:500) and labelled with FITC labelled secondary antibody (1:50). Pre-immune serum or an isotype-matched control antibody was used as a control.*

ual colonies appeared which were picked and plated into wells of a 24-well plate. When confluent, the cells were screened for surface expression of MHC class I molecules using the monoclonal antibody W6/32 (Serotec, Oxford, UK) by flow cytometry. Cells were routinely grown in MEMα supplemented with FCS and geneticin to ensure propagation of A2RSV5neo transformants. A control cell line was derived that had been stably transfected with RSVneo and selected with geneticin.

Flow cytometry

Immunolabelling was performed using standard techniques. Briefly, cells were harvested by treatment with trypsin and incubated with saturating quantities of primary antibody for 30 min at 4°C and washed twice in an excess of PBS. Cells were then incubated with fluorochrome conjugated secondary antibody for 30 min at 4°C and then washed twice with PBS. Samples were analysed immediately on a FACSCalibre flow cytometer (Becton Dickinson, Oxford, UK) with viability measurements taken concurrently using propidium iodide as a non-specific stain.

Expression and purification of fibre knob

The knob domain of the Ad5 fibre was expressed in *E. coli* with an N-terminal hexahistidine tag. The knob domain and the last repeat of the shaft domain of the fibre were obtained by PCR amplification using wild-type Ad5 DNA as template and oligonucleotides designed to permit ligation of the PCR product into the bacterial expression vector pET-28b (Novagen; Cambridge Bioscience, Cambridge, UK). The primers used were: forward primer; 5′-CGG CAT ATG GGT GCC ATT ACA GTA GGA AAC and reverse primer; 5′-CCC GTC GAC TGT GTT GGG AGG GAG GTG GAC GG. The PCR product was digested with *Nde*I and *Sal*I and cloned into *Nde*I–*Sal*I digested pET28b. The fibre knob pET-28b plasmid was transformed into the *E. coli* strain BL21 (DE3) (Novagen; Cambridge Bioscience) and IPTG was used to induce expression of the hexahistidine fusion protein. The resulting protein was purified from the soluble fraction of the bacterial cell lysate by binding to TALON metal chelating resin (Clontech, Basingstoke, UK). The fusion protein was eluted with 150 mm imidazole and the His tag removed by cleavage with thrombin (Sigma), yielding a knob protein that was electrophoretically homogeneous as judged by SDS-PAGE. Gel filtration analysis on a Fast Protein Liquid Chromatography (FPLC) system using a Superose 12 column (Amersham-Pharmacia, Little Chalfont, UK) and molecular weight standards showed the truncated fibre to be trimeric.

Iodination of fibre knob

The fibre knob (20 μg, purified by FPLC) in 10 mm sodium phosphate buffer (pH 8.2) was incubated with 1 mCi of ^{125}I-labelled Bolton-Hunter reagent (Dupont NEN, Hounslow, UK) on ice for 3 h. Iodinated fibre was then purified through a PD-10 column (Amersham-Pharmacia) pre-equilibrated in PBS containing 1% gelatin. The specific activity of the ^{125}I-labelled truncated fibre protein was routinely between 1600–2100 Ci/mmol. The trimeric nature of the labelled protein was verified by analysis of its thermal stability by SDS-PAGE.[18]

Receptor binding assays

Cells were grown in DMEM supplemented with 10% FCS and plated in 24-well tissue culture plates. Confluent monolayers were then washed twice with binding buffer (Hepes buffered DMEM (pH 7.2), 0.1% BSA) and incubated at 4°C for 8 h in the same buffer containing increasing concentrations of ^{125}I-fibre knob in a final volume of 200 μl. Free ligand was removed by washing the cells

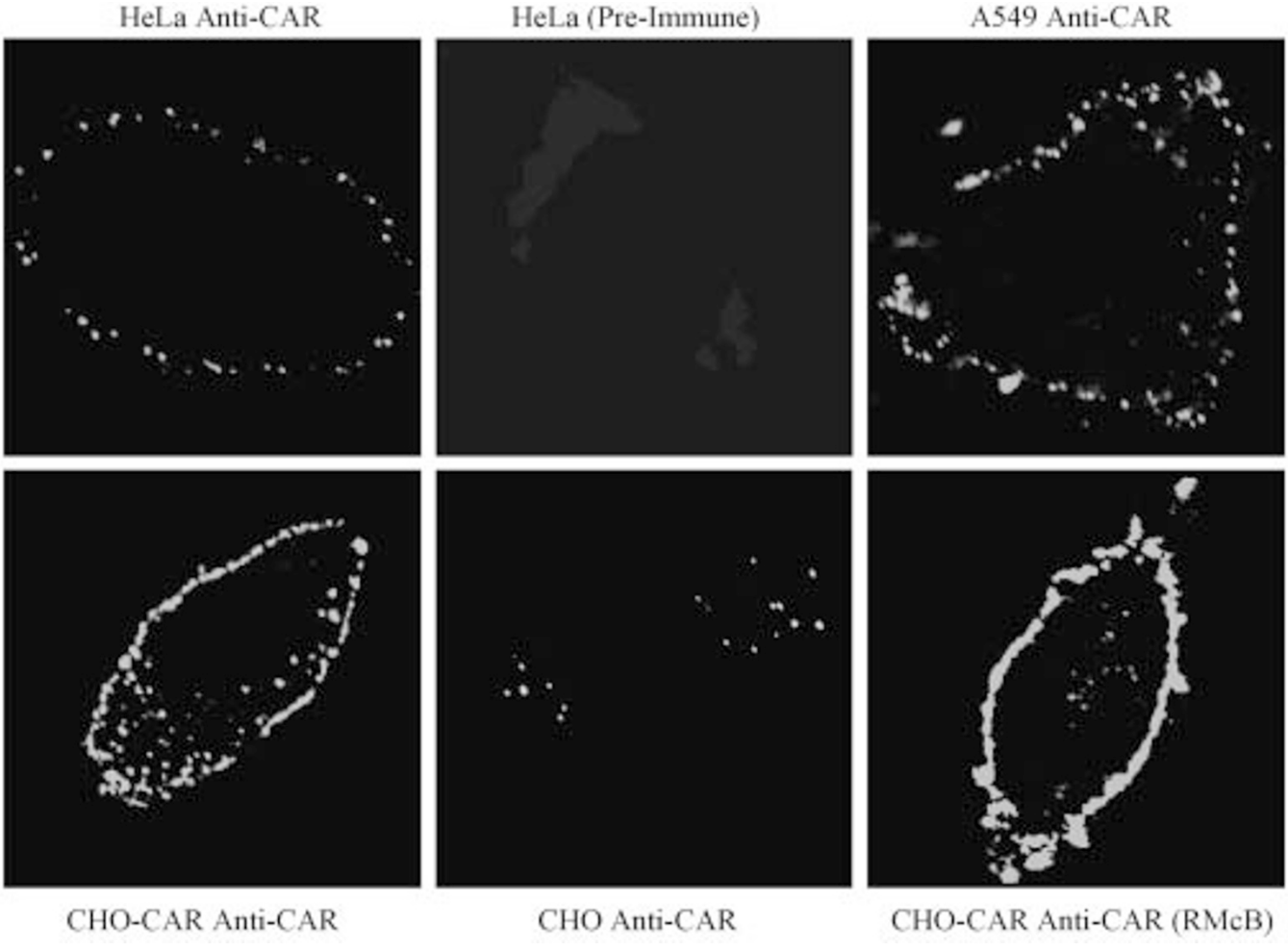

Figure 4 *Plasma membrane localisation of CAR. Cells were fixed with 4% formaldehyde and labelled with rabbit anti-CAR (1:100) or RmcB (1:500) visualised with FITC-labelled secondary antibody and viewed by laser deconvolution microscopy.*

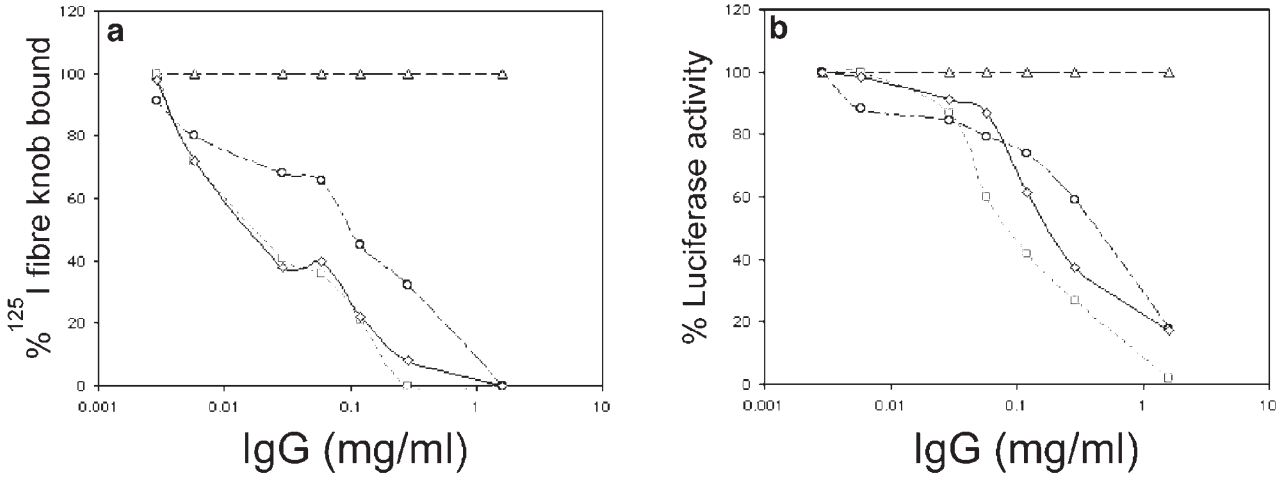

Figure 5 *Inhibition of Ad5 fibre binding and virus uptake by the anti-CAR antibody. Cell monolayers in 24-well plates were incubated with serial dilutions of the IgG fraction of polyclonal anti-CAR and 100 Pm ^{125}I fibre knob (a) or Ad5luc3 (b). For ligand binding studies, cells were incubated at 4°C for 8 h and cell-bound radioactivity at each concentration of anti-CAR was plotted as a percentage of total bound in the absence of anti-CAR. Ad5luc3 was incubated in the presence of serial dilutions of anti-CAR at 4°C for 1 h. After washing, cells were incubated at 37°C for 18 h. Luciferase expression is expressed as a percentage of control (ie in the absence of antibody). Rabbit IgG against human BSA was used as a control with A549 cells. □, A549; ◇, HRT 18; ○, Fen 2; △, Control.*

three times with ice-cold PBS. Cells were then solubilised with 0.1 N NaOH and counted in a Wizard gamma counter (LKB, Bromma, Sweden). Non-specific binding was defined as cell-associated radioactivity in the presence of saturating concentrations of unlabelled fibre knob (10^{-7} m) and was always <5% of total binding. Results were analysed by computerised model fitting using the ORIGIN program (MICROCAL) to calculate K_d.

Cloning and expression of the CAR extracellular domain

The extracellular domain of CAR was amplified from A549 cDNA using the proof reading enzyme *Pfu* DNA polymerase (Stratagene) and subcloned into pIBI30 (International Biotechnologies, Cambridge, UK). Total RNA was isolated from confluent monolayers using the Trizol reagent (Clontech) and DNase I treated at 37°C for 30 min. cDNA was prepared from total RNA using oligo(dT) primers. PCR primers were designed from the published cDNA sequence of the human CAR receptor[6] (forward primer; 5′-GG CAT ATG GAT TTC GCC AGA AGT TTG; reverse primer: 5′-CGA GTC GAC TTA AGC TTT ATT TGA AGG AGG) and PCR performed for 40 cycles (95°C for 1 min, 49°C for 1 min, 72°C for 2 min; final extension 72°C for 10 min). Sequencing of the resulting cloned cDNA demonstrated that the sequence was identical to the published human CAR sequence.[6] The cloned fragment was excised from the plasmid with *Nde*I and *Sal*I and cloned into the bacterial expression vector, pET28b (Novagen; Cambridge Bioscience). The resulting recombinant plasmid was transformed into the *E. coli* strain BL21 (DE3). The hexahistidine fusion protein was extracted from the insoluble fraction of the bacterial cell lysate by solubilisation with sarkosyl and purified by binding to TALON metal chelating resin (Clontech).

Preparation and purification of anti-CAR polyclonal antibody

Rabbits were injected subcutaneously with 25 μg of recombinant CAR protein in Freund's complete adjuvant (Sigma). After 10 days the injection was repeated with CAR mixed with Freunds Incomplete Adjuvant (Sigma, Poole, UK). This latter injection was repeated three times at 10-day intervals, at which time serum was obtained. For inhibition of fibre binding and viral uptake, the IgG fraction of the rabbit serum was purified on a Protein A affinity column (Amersham-Pharmacia). The IgG fraction was eluted in 0.1 m glycine, dialysed into PBS and concentrated using a C-10 Amicon concentrator (Millipore, Watford, UK).

Immunofluoresence using the polyclonal anti-CAR antibody

Cells were grown on glass cover slips, washed once in PBS and fixed in 4% formaldehyde for 10 min at room temperature. The cells were washed extensively with PBS and incubated with anti-CAR (1:100) or the mouse monoclonal antibody against human CAR[6] (RmcB, 1:500) for 1 h at 37°C. Cells were washed six times with PBS and incubated for 30 min at 37°C in a 1:1000 dilution of a goat anti-mouse or goat anti-rabbit FITC-conjugated secondary antibody (Sigma). Fluorescence microscopy was performed using a digital imaging system and Openlab computer software (Improvision, Coventry, UK). Optical sections of cell samples were obtained by laser deconvolution microscopy.

Antibody blocking of fibre knob binding

Confluent monolayers of cells were incubated with ^{125}I fibre knob and varying amounts of purified IgG fraction of the rabbit anti-CAR in 24-well tissue culture plates. Incubations were carried out for 8 h at 4°C and binding was assessed as described above.

Viruses and uptake of virus into cells

The Ad5CMVEGFP virus is replication-deficient and contains an enhanced GFP cDNA under the control of the CMV promoter in the viral E1 region (A Tolkovsky and M Roberts, personal communication) whereas Ad5luc3 is replication-competent and contains the luciferase coding region cloned in the E3 region.[19] The viruses were propagated in 293 and HeLa cells, respectively, and purified as described.[20] The titre of the viral stock was determined by fluorescent focus unit (FFU) assay in 293 cells.[20] Cells were plated in 24-well plates 1 day before infection and pre-equilibrated to 4°C, 30 min before use. Ad5CMVEGFP was diluted in Hepes buffered DMEM containing 4% FCS and added directly to the CHO cell lines at a multiplicity of infection of 100 FFU per cell. After incubation for 1 h at 4°C the monolayers were washed three times with DMEM containing 2% FCS to remove unbound virus.[11] DMEM containing 2% FCS prewarmed to 37°C was added and the cells incubated for 20 h at 37°C. Cells were trypsinised and GFP expression measured using flow cytometry. For antibody inhibition assays Ad5luc3 (at a multiplicity of infection of 100 FFU per cell) was added to a dilution series of anti-CAR or control antibody (IgG fraction of polyclonal rabbit anti-human BSA; Sigma) in Hepes buffered DMEM containing 4% FCS and incubated initially at 4°C as described above. After 24 h at 37°C the cells were scraped from individual wells and aliquots assayed for luciferase activity according to the manufacturer's instructions (Promega, Southampton, UK). Protein was estimated by the BCA method (Pierce and Warriner, Chester, UK).

Acknowledgements

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References

1 Smith AE. Viral vectors for gene therapy. *Ann Rev Microbiol* 1995; **49**: 807–838.
2 Freytag SO *et al*. A novel three-pronged approach to kill cancer cells selectively: concomitant viral, double suicide gene and radiotherapy. *Hum Gene Ther* 1998; **9**: 1323–13333.
3 Philipson L, Lonberg-Holm K, Pettersson U. Virus–receptor interaction in the adenovirus system. *J Virol* 1968; **2**: 1064–1075.
4 Stevenson SC *et al*. Human adenovirus serotypes 3 and 5 bind to two different cellular receptors via the fiber head domain. *J Virol* 1995; **69**: 2850–2857.
5 Wickham TJ *et al*. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ promote adenovirus internalisation but not virus attachment. *Cell* 1993; **73**: 309–319.
6 Bergelson JM *et al*. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997; **275**: 1320–1323.
7 Tomko RP, Xu R, Philipson L. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. *Proc Natl Acad Sci USA* 1997; **94**: 3352–3356.

8 Hidaka C *et al*. CAR-dependent and CAR-independent pathways of adenovirus vector-mediated gene transfer and expression in human fibroblasts. *J Clin Invest* 1999; **103**: 579–587.
9 Kaner RJ *et al*. Modification of the genetic program of human alveolar macrophages by adenovirus vectors *in vitro* is feasible but inefficient, limited in part by the low level of expression of coxsackie/adenovirus receptor. *Am J Respir Cell Mol Biol* 1999; **20**: 361–370.
10 Leon RP *et al*. Adenoviral-mediated gene transfer in lymphocytes. *Proc Natl Acad Sci USA* 1998; **95**: 13159–13164.
11 Hong SS *et al*. Adenovirus type 5 fiber knob binds to MHC class I α2 domain at the surface of human epithelial and B lymphoblastoid cells. *EMBO J* 1997; **16**: 2294–2306.
12 Gillott DJ *et al*. Accurate and rapid assessment of MHC antigen upregulation following cytokine stimulation. *J Immunol Meth* 1993; **165**: 231–239.
13 Wildner O, Blaese RM, Morris JC. Therapy of colon cancer with oncolytic adenvirus is enhanced by addition of herpes simplex virus-thymidine kinase. *Cancer Res* 1999; **59**: 410–413.
14 Heise C *et al*. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and anti-tumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nature Med* 1997; **3**: 639–645.
15 Garrido F *et al*. HLA class I antigen in human tumors. *Adv Cancer Res* 1995; **67**: 155–195.
16 Hemmi S *et al*. The presence of human coxsackie and adenovirus receptor is associated with efficient adenovirus-mediated transgene expression in human melanoma cell cultures. *Hum Gene Ther* 1998; **9**: 2363–2373.
17 Long EO *et al*. Efficient cDNA expression vectors for stable and transient expression of HLA-DR in transfected fibroblast and lymphoid cells. *Hum Immunol* 1991; **31**: 229–235.
18 Michael SL *et al*. Addition of a short peptide ligand to the adenovirus fiber protein. *Gene Therapy* 1995; **2**: 660–668.
19 Mittal SK *et al*. Monitoring foreign gene expression by a human adenovirus-based vector using the firefly luciferase gene as a reporter. *Virus Res* 1993; **28**: 67–90.
20 Tollefson AE *et al*. Preparation and titration of CsCl-banded adenovirus stock. In: Wold WSM (ed). *Methods in Molecular Medicine, Vol 21: Adenovirus Protocols and Methods*. Humana Press: Totawa, NJ, 1998, pp 1–9.