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Efficient gene transfer into various mammalian cells, including non-hepatic cells, by baculovirus vectors

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A baculovirus (*Autographa californica* nucleopolyhedrovirus) vector containing a strong promoter, the CAG promoter, was developed to introduce foreign genes into mammalian cells. Recombinant baculoviruses carrying a reporter gene under the control of the CAG promoter were inoculated into various mammalian cell lines. High-level expression was observed not only in hepatocytes but also in other non-hepatic cell lines tested. Expression of the reporter gene was detected even 14 days after infection. The infectious titre of the recovered baculoviruses decreased significantly after infection, indicating that the baculoviruses did not replicate in mammalian cells. We then compared the efficiencies of gene expression by the baculovirus vector with that of a replication-defective adenovirus vector by using the same expression unit. The same level of expression was observed in HepG2, HeLa and COS7 cells by both vectors. Efficient expression and proper processing were observed in mammalian cells infected with baculoviruses carrying genes coding for structural regions of hepatitis C virus. These results suggest that the baculovirus vector is a good tool for gene delivery into various mammalian cells in order to study the function of foreign genes.

Introduction

The introduction of foreign genes into cultured mammalian cells or tissues is essential for understanding the function of genes and their products. Transfection, electroporation, direct injection and viral vectors have been used to transfer genes of interest into targeted cells. A new trend for gene therapy has emphasized the further need for vectors that can efficiently deliver foreign genes into mammalian cells (Anderson, 1992; Mulligan, 1993).

A baculovirus [*Autographa californica* nucleopolyhedrovirus (AcNPV)] expression system has been used for expression of a wide variety of foreign genes because of its high-level expression in insect cells (Luckow & Summers, 1988; Smith *et al.*, 1983). Its host specificity has long been considered to be restricted to cells derived from arthropods. AcNPV was studied in the past with regard to its ability to infect mammalian cells (Carbonell *et al.*, 1985). Recently, Hofmann *et al.* (1995) have shown that AcNPV can infect human hepatocytes and that a reporter gene, the luciferase gene, is highly expressed from the cytomegalovirus (CMV) immediate early (IE) promoter. Boyce & Bucher (1996) have also shown that a human liver tumour line, HepG2, and primary rat hepatocytes are able to efficiently express a different reporter gene, the *lacZ* gene, under the control of the Rous sarcoma virus (RSV) promoter inserted into AcNPV. In these two studies, high-level expression was achieved only in hepatocytes but not in other epithelial cells.

When viral vectors are utilized *in vivo* and *in vitro*, virus replication sometimes makes it difficult to analyse the function of foreign genes. Therefore it is important to investigate virus replication in cells inoculated with viral vectors.

In this study, we have characterized a baculovirus vector carrying a foreign gene under the control of the CAG promoter in comparison with a replication-defective adenovirus vector. Results suggest that the baculovirus vector is a suitable system

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to study the function of foreign genes in various mammalian cells without effects of virus replication.

Methods

■ **Construction of plasmids.** Recombinant baculoviruses were constructed by use of the transfer vector pAcYM1 (Matsuura *et al.*, 1987). To express foreign genes in mammalian cells, we utilized the CAG promoter, a composite promoter consisting of the CMV IE enhancer, chicken β-actin promoter and rabbit β-globin polyadenylation signal (Niwa *et al.*, 1991). The CAG promoter can be utilized in a wide variety of mammalian cell lines and exhibits stronger expression than the CMV promoter and the RSV promoter (Niwa *et al.*, 1991). To examine expression of foreign genes in mammalian cells, the *Photinus pyralis* luciferase gene (De Wet *et al.*, 1987) or *Escherichia coli lacZ* gene was introduced into the baculovirus vector under the CAG promoter. pCAGwt (Kanegae *et al.*, 1995) is a derivative of pCAGGS (Niwa *et al.*, 1991) containing the unique *Swa*I site. To generate pCAGluc, the luciferase gene was excised from plasmid PG-CS (Toyo Ink, Tokyo, Japan) with *Bam*HI and *Xho*I, filled in with Klenow enzyme, and inserted into the *Swa*I site of pCAGwt. To remove the polyhedrin promoter from pAcYM1, the transfer vector was digested with *Eco*RV and *Bam*HI and filled in with Klenow enzyme. A CAG-luc cassette was excised from pCAGluc by use of *Sal*I and *Hin*dIII and filled in with Klenow enzyme. To construct pAcCAluc (Fig. 1), the CAG-luc cassette was inserted into the modified pAcYM1. A CAG-*lacZ* cassette was excised from pCA*lacZ* (Y. Kanegae and others, unpublished data) by use of *Sal*I and *Hin*dIII and filled in with Klenow enzyme. To construct pAcCA*lacZ* (Fig. 1), the CAG-*lacZ* cassette was inserted into the modified vector.

To compare the efficiencies of gene expression by baculovirus harbouring the CAG promoter with expression under the CMV promoter, transfer vector pAcCMVluc carrying the luciferase gene under the CMV promoter was constructed as follows. A cDNA for the CMV promoter was excised from pcDNA3 (Invitrogen) by digestion with *Nae*I, filled in with Klenow enzyme, ligated with a p*Bcl*I linker (5′ CTGATCGA 3′) (TOYOBO, Tokyo, Japan), and then digested with *Bcl*I and *Nru*I. The DNA fragment for the CMV promoter was inserted into the modified pAcYM1 and the luciferase gene was inserted under the CMV promoter.

Transfer vectors, pAcCAGMCS1 and pAcCAGMCS2, harbouring the CAG promoter and multicloning sites, were constructed for subcloning of HCV cDNAs. To generate recombinant baculoviruses for expression of hepatitis C virus (HCV) structural proteins in mammalian cells, two transfer vectors were constructed. AcCA39 carries the region for HCV core protein and pAcCA327 carries the region for HCV core-E1-E2. HCV cDNAs within pAcCA39 and pAcCA327 correspond to amino acids 1–194 (Takeuchi *et al.*, 1990; Suzuki *et al.*, 1995) and 1–810 (Matsuura *et al.*, 1994), respectively.

■ **Cells and viruses.** AcNPV and recombinant baculoviruses were grown and assayed in *Spodoptera frugiperda* (Sf) 9 cells in TC100 medium (GIBCO) supplemented with 0·26% Bacto tryptose phosphate broth (Difco), 100 μg/ml kanamycin and 10% foetal bovine serum. Recombinant baculoviruses were generated by homologous recombination, as described previously (Matsuura *et al.*, 1987), and purified as follows; culture supernatants were harvested at 3 days after infection, and cell debris was removed by centrifugation at 6000 ***g*** for 15 min at 4 °C. Virus was pelleted by ultracentrifugation at 25 000 r.p.m. in an SW28 rotor (Beckman) for 90 min and resuspended in 1 ml of PBS, loaded on 10–60% (w/v) sucrose gradients, and ultracentrifuged at 25 000 r.p.m. in an SW41E rotor (Beckman) for 90 min. The virus band was collected and resuspended in PBS and ultracentrifuged at 25 000 r.p.m. in the SW41E rotor for 90 min. The virus pellet was resuspended in PBS and infectious titres were determined by plaque assay.

To compare the efficiencies of gene expression of the baculovirus vector with the replication-defective adenovirus vector, the adenovirus expressing the *lacZ* gene under the CAG promoter, AdexCA*lacZ*, was utilized (Fujita *et al.*, 1995). The recombinant adenovirus was titrated by an endpoint of cytopathic effects (CPE) method. One $TCID_{50}$/ml corresponds to approximately 1 p.f.u./ml (Kanegae *et al.*, 1994).

Various mammalian cell lines were used to examine gene expression by the recombinant baculoviruses, including human cell lines (HepG2, Huh7, HeLa, KATO-III, IMR32 and MT-2 cells), a monkey kidney cell line (COS7), porcine kidney cell lines (CPK and FS-L3), a rat gastric mucosal cell line (RGM1) and a rat pheochromocytoma line (PC12). These cells were grown in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) containing 2 mM L-glutamine, penicillin (50 IU/ml), streptomycin (50 mg/ml) and 10% foetal bovine serum or in the media recommended by the ATCC. Foetal bovine serum was treated at 56 °C for 30 min. HepG2 was purchased from the Dainippon Pharmaceutical Co., Osaka. Huh7 was a gift from the Japanese Cancer Research Resourses Bank (JCRB)-Cell, Tokyo. RGM1 is available from the Riken Cell Bank, Tsukuba, Japan. CPK (Komaniwa *et al.*, 1981) and FS-L3 (Sakoda *et al.*, unpublished data) were provided by A. Fukusho, National Institute of Animal Health, Tokyo, Japan.

■ **Luciferase assays.** Cells were infected with AcCAluc or AcCMVluc at an indicated m.o.i. The viruses were adsorbed to the cells at 37 °C for 1 h. Extracts were prepared from cells harvested at 48 h after infection. Luciferase activity was determined by use of the Pica Gene luciferase assay kit (Toyo Ink, Tokyo, Japan), according to the manufacturer's instructions. Cells were lysed with the cell lysis buffer LUC/PGC-50 (Toyo Ink). Twenty μl of cleared cell lysate was incubated with 100 μl of a reaction mixture containing 137 mM NaCl, 2·7 mM KCl, 4·3 mM Na_2HPO_4, 1·4 mM KH_2PO_4, 20 mM Tricine, 1·07 mM $(MgCO_3)_4Mg(OH)_2.5H_2O$, 2·67 mM $MgSO_4$, 0·1 mM EDTA, 33·3 mM DTT, 270 μM Coenzyme A, 470 μM luciferin and 530 μM ATP. Relative light units (rlu) were measured by use of a luminometer (Berthold).

■ **β-Galactosidase assays.** Levels of β-galactosidase were measured by a biochemical assay with *o*-nitrophenyl β-D-galactopyranoside (Nielsen *et al.*, 1983). We used the β-galactosidase assay kit (Stratagene) according to the manufacturer's instructions. Cells were infected with AcCA*lacZ* or AdexCA*lacZ* at m.o.i. values of 1, 10 and 100, respectively. Cell extracts were prepared at 48 h after infection. Protein was quantified by use of the BCA protein assay reagent (Pierce). β-Galactosidase activities were expressed as units (nmol *o*-nitrophenyl β-D-galactopyranoside cleaved/min)/mg protein. The β-galactosidase activity was calculated according to the manufacturer's instructions.

For *in vivo* staining of β-galactosidase activity, at 48 h after infection, the cells were washed twice with PBS, fixed with 0·25% glutaraldehyde and stained with a solution of 1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal), 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$ and 2 mM $MgCl_2$ in PBS (Schöler *et al.*, 1989).

■ **Western blotting.** Cell extracts were separated by SDS–PAGE, and proteins were blotted to a polyvinylidene difluoride membrane (Millipore). The immunoblot analysis was carried out as described previously (Harada *et al.*, 1991).

■ **Antibodies.** To examine expression of HCV core protein, an anti-

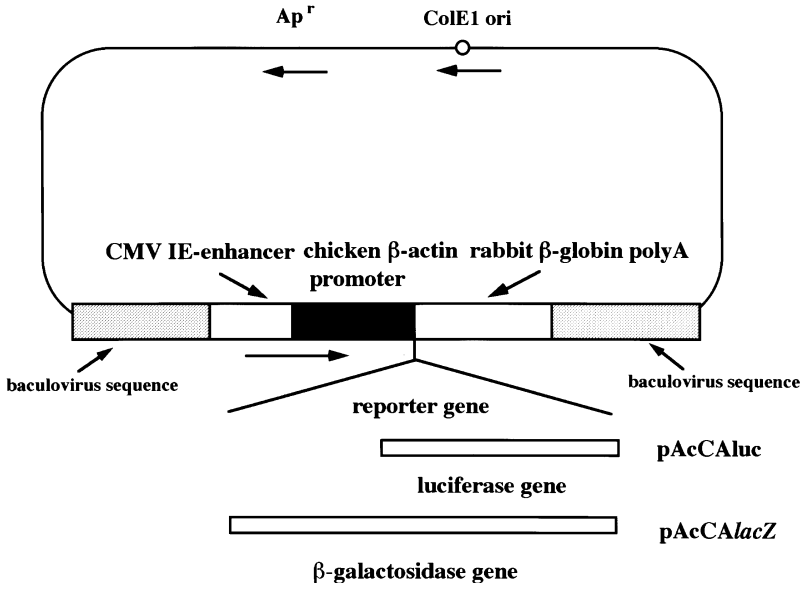

Fig. 1. Construction of transfer vectors to generate recombinant baculoviruses AcCAluc and AcCA*lacZ*. The CAG promoter is composed of the CMV immediate early enhancer, chicken *β*-actin promoter and rabbit *β*-globin polyadenylation signal. Transfer vector pAcCAluc: the *Photinus pyralis* luciferase gene was inserted under the control of the CAG promoter. Transfer vector pAcCA*lacZ*: the *Escherichia coli lacZ* gene was inserted under the control of the CAG promoter.

HCV core protein mouse monoclonal was used as the first antibody (Suzuki *et al*., 1995).

Results

Susceptibility of various cell lines to a recombinant baculovirus

To examine the susceptibility of various mammalian cell lines to baculovirus-mediated gene transfer, we utilized the recombinant baculovirus AcCAluc carrying the luciferase gene under the CAG promoter. Expression from AcCAluc in various mammalian cell lines was determined by luciferase assay of extracts prepared from infected cells. Mock-infected cells were used as a control of background luciferase activity. AcCAluc used for inoculation was extensively purified by ultra-centrifugation and no luciferase activity was detected in the inocula (data not shown). We could observe high luciferase activity not only in human hepatocytes but also in other mammalian cells (Fig. 2). The human hepatocellular carcinoma cell lines HepG2 and Huh7, porcine kidney cell line CPK and monkey kidney cell line COS7 exhibited high level expression of luciferase. We detected significant levels of luciferase expression in the human cervix carcinoma cell line HeLa, porcine kidney cell line FS-L3 and human gastric cancer line KATO-III. Low-level expression was found in the other cell lines, RGM1, PC12, IMR32 and MT-2 cells.

Comparison of luciferase expression by AcCAluc and AcCMVluc

To examine promoter dependency of gene expression by baculovirus vectors in mammalian cells, we compared luciferase expression driven from the CAG promoter with that driven from the CMV promoter. HepG2, Huh7, HeLa and COS7 cells were infected with AcCAluc or AcCMVluc at an m.o.i. of 20 (Fig. 3). Human hepatocellular carcinoma lines, HepG2 and Huh7, and COS7 cells showed high levels of luciferase activity by both vectors. On the other hand, HeLa cells infected with AcCAluc exhibited more than 10-fold higher luciferase activity than those infected with AcCMVluc.

Comparison of the baculovirus vector with the replication-defective adenovirus vector

To investigate the efficiencies of baculovirus-mediated gene transfer more precisely, we compared the efficiency of gene expression by the baculovirus vector with that of the replication-defective adenovirus vector by use of the same expression unit (*β*-galactosidase gene under the control of the CAG promoter) in various mammalian cell lines. Cells were infected with the recombinant baculovirus AcCA*lacZ* or the recombinant adenovirus AdexCA*lacZ* m.o.i. values of 1, 10 and 100. *β*-Galactosidase activity increased in a dose-dependent manner in both systems. All of the cell lines infected with AdexCA*lacZ* or AcCA*lacZ* showed high-level *β*-galactosidase activity (Fig. 4). HepG2 cells infected with AcCA*lacZ* at an m.o.i. of 100 showed higher *β*-galactosidase activity than those infected with AdexCA*lacZ*. HepG2 cells infected with AcCA*lacZ* showed less cytotoxicity even at a higher m.o.i. than those infected with AdexCA*lacZ* (data not shown). HeLa and COS7 cell lines infected with AcCA*lacZ* or AdexCA*lacZ* showed similar *β*-galactosidase activity.

The cells were then stained with X-Gal to determine efficiencies of gene transfer by both vectors. No or very few stained cells were found in mock-infected preparations. The proportion of stained cells increased in a dose-dependent manner according to the m.o.i. of the recombinant viruses carrying the *lacZ* gene with both vectors (data not shown).

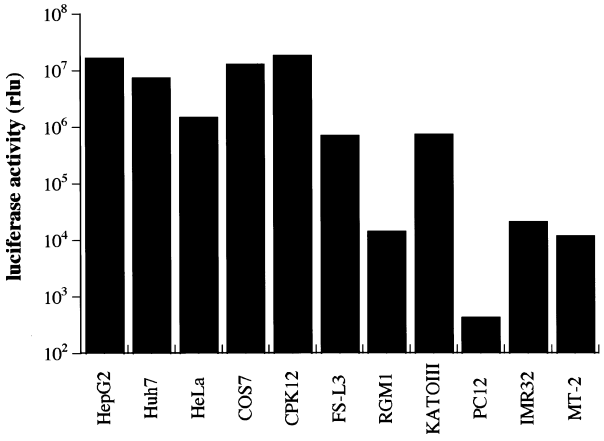

Fig. 2

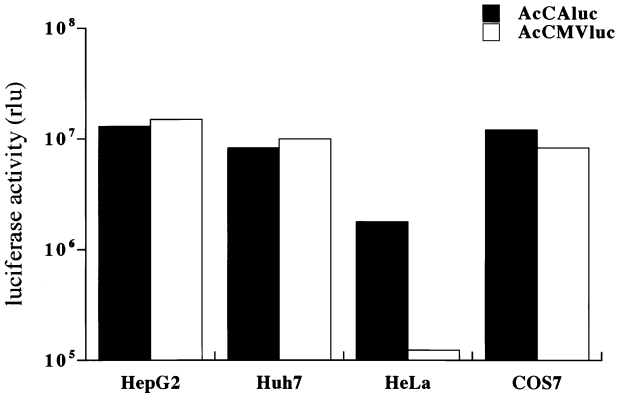

Fig. 3

Fig. 2. Expression of the luciferase gene in various mammalian cells infected with AcCAluc. Cells were infected with AcCAluc at an m.o.i. of 20 and harvested at 48 h after infection. Luciferase activity was measured as described in Methods. Values for relative light units (rlu) refer to extracts from 10^5 cells. The value for mock-infected cells was subtracted in all cases. The results shown are representative of three independent experiments.

Fig. 3. Luciferase activity in cells infected with AcCAluc or AcCMVluc. Cells were infected with AcCAluc or AcCMVluc at an m.o.i. of 20 and harvested at 48 h after infection. Luciferase activity was measured as described in Methods. Values for relative light units (rlu) refer to extracts from 10^5 cells. The value for mock-infected cells was subtracted in all cases. The results are representative of three independent experiments.

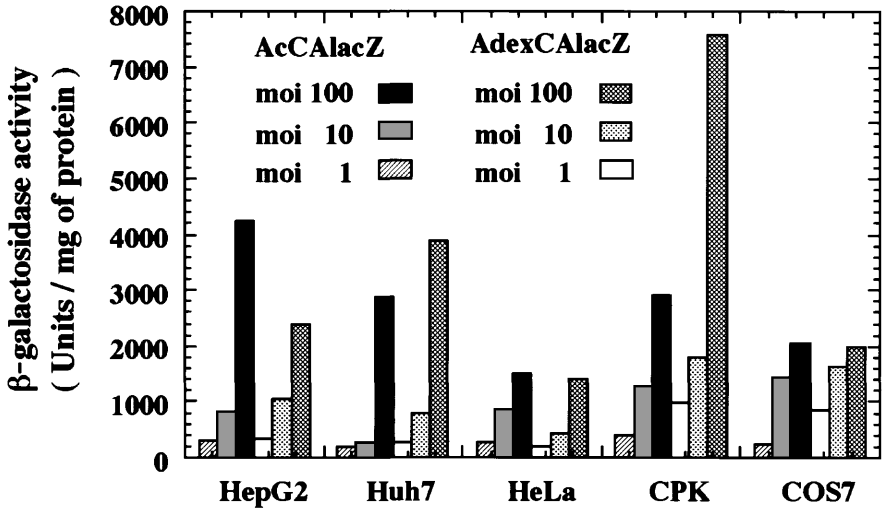

Fig. 4. Expression of the *lacZ* gene by the recombinant baculovirus AcCA*lacZ* and the recombinant adenovirus AdexCA*lacZ*. Cells were infected with AcCA*lacZ* or AdexCA*lacZ* at m.o.i. values of 1, 10 and 100. Cells were harvested at 48 h after infection. β-Galactosidase activity was measured as described in Methods. Values are expressed as units of β-galactosidase activity/mg protein. The value for mock-infected cells was subtracted in all cases. The results shown are representative of three independent experiments.

Although the intensity of staining by X-Gal was higher in most of the cell lines infected with the adenovirus than with the baculovirus, in HepG2, Huh7, CPK, HeLa and COS7 cells infected with AcCA*lacZ* at an m.o.i. of 100, 90–100% of the cells were stained by X-Gal (Fig. 5). This result indicates that the reporter gene was transferred and expressed efficiently in these cultured mammalian cells by the baculovirus vector. Especially in HepG2 and CPK, the efficiencies were very high and more than 80% of the cells were stained by X-Gal even at an m.o.i. of 10 (data not shown).

Most of the cell lines inoculated with the adenovirus exhibited apparent CPE. In contrast, no visible CPE were detected in the cell lines inoculated with the baculovirus even at a high m.o.i., suggesting much lower cytotoxicity than with the adenovirus (data not shown).

Titration of infectious baculovirus after inoculation

HepG2 cells were infected with AcCAluc at an m.o.i. of 20. After 1 h, they were extensively washed with fresh medium and further incubated at 37 °C. The cells were harvested at various times. Infectious titres of the virus recovered from HepG2 cells were measured by a plaque assay to determine whether the baculovirus can replicate in mammalian cells or not. As shown in Fig. 6, the virus titres decreased immediately after infection. The titres of the virus recovered at 48 h post-

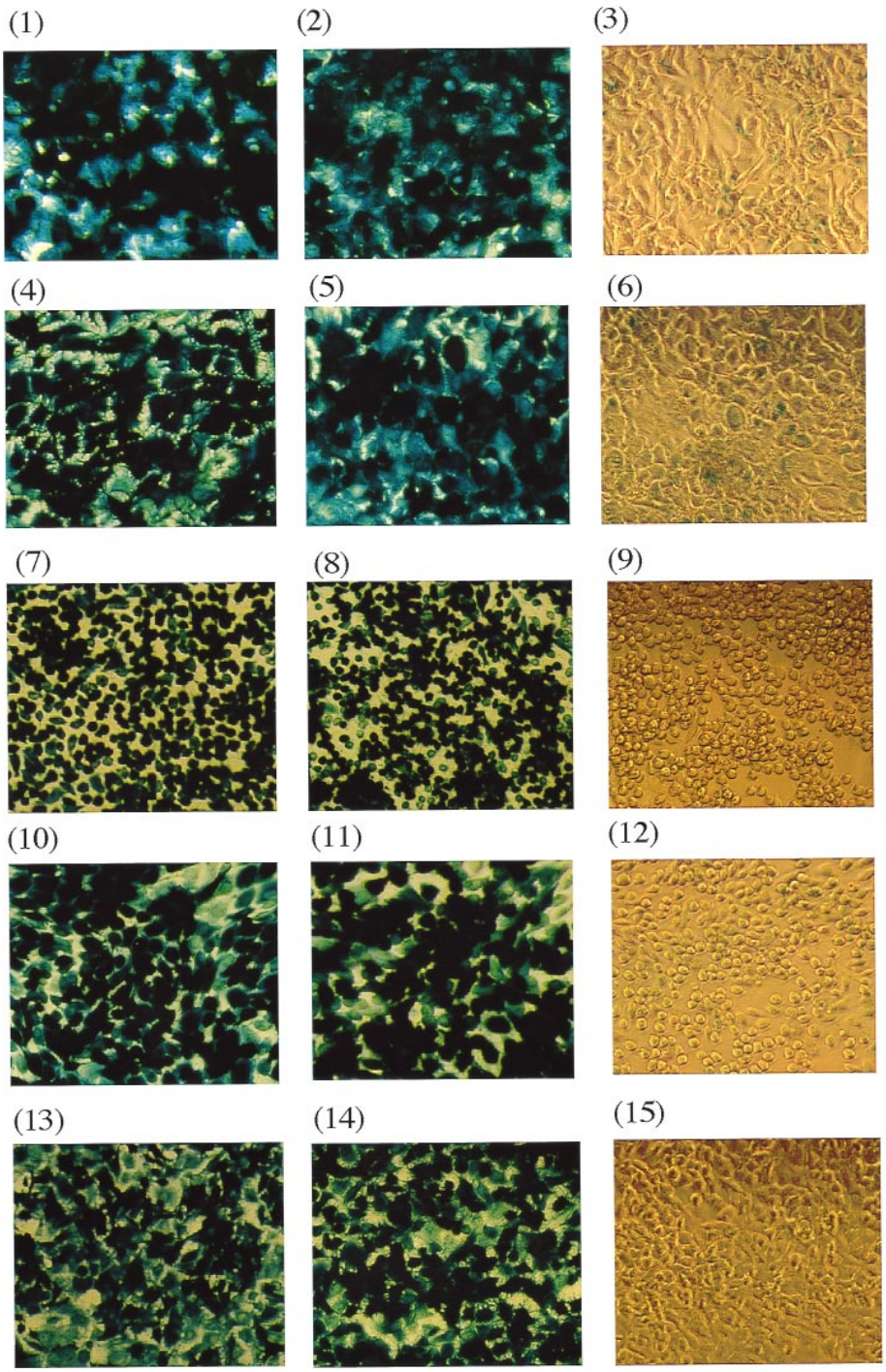

Fig. 5. X-Gal staining of cells infected with the recombinant baculovirus AcCA*lacZ* or the recombinant adenovirus AdexCA*lacZ*. Cells were stained with X-Gal at 48 h after infection with the recombinant baculovirus AcCA*lacZ* or the recombinant adenovirus AdexCA*lacZ*. 1–3, HepG2 cells; 4–6, Huh7 cells; 7–9, HeLa cells; 10–12, CPK cells; 13–15, COS7 cells. 1, 4, 7, 10 and 13, cells infected with AcCA*lacZ* at an m.o.i. of 100; 2, 5, 8, 11 and 14, cells infected with AdexCA*lacZ* at an m.o.i. of 100; 3, 6, 9, 12 and 15, mock-infected cells.

infection was 100-fold lower than those recovered at 1 h post-infection. No infectious virus was recovered at 9 days after inoculation. The result indicates that the baculovirus did not replicate in HepG2 cells. Luciferase activity was detected as early as 1 h post-infection and reached the highest level at 24–48 h post-infection. Although the recovered virus titres

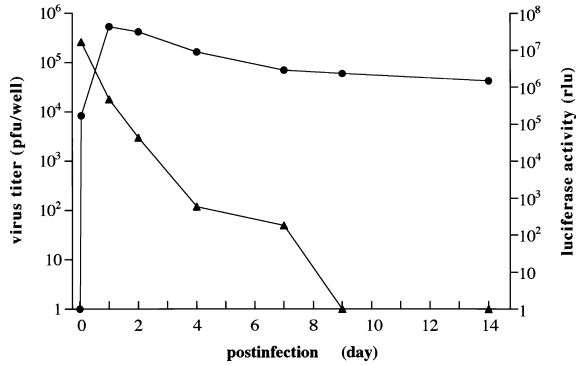

Fig. 6. Titration of infectious baculovirus AcCAluc. HepG2 cells in 12-well plates were infected with AcCAluc at an m.o.i. of 20. After adsorption for 1 h, cells were extensively washed with fresh medium and incubated with DMEM supplemented with 2% foetal bovine serum. This time is designated 1 h post-infection. The time-point taken prior to infection is defined as zero time. Cells and supernatant were harvested at the indicated times. The sample was sonicated, cell debris was removed by centrifugation and the final supernatant inoculated into Sf9 cells. Infectious titres (▲) were determined by plaque assay. Luciferase activity (●) was measured as described in Methods. pfu, plaque forming unit. The results shown are representative of three independent experiments.

decreased significantly after infection, expression of luciferase continued and the enzyme activity was detected even at 14 days post-infection.

Expression of HCV structural proteins in mammalian cells by recombinant baculoviruses

In previous studies, we showed that HCV structural protein is processed by cellular signalase(s) (Matsuura *et al.*, 1994). To determine whether HCV proteins are processed properly in this system, we constructed baculoviruses carrying the gene coding for structural proteins of HCV under the CAG promoter. We utilized two recombinant baculoviruses, AcCA39 and AcCA327, carrying HCV core protein and all of the structural proteins, respectively. Expression and processing of HCV proteins were examined in HepG2, Huh7, HeLa, CPK and COS7 cells. To examine expression of the HCV core protein in this system, the cell extracts obtained at 48 h after infection were analysed by Western blotting by use of the monoclonal antibody raised against HCV core protein. Sf9 cells were infected with the recombinant baculovirus Ac39 or Ac327 as a positive control (Suzuki *et al.*, 1995; Matsuura *et al.*, 1994). When either AcCA39 or AcCA327 was inoculated, 22 kDa HCV core protein was observed in each of the cell lines examined (Fig. 7). No band was detected in the mock-infected cells. We could also observe 35 kDa E1 protein and 60 kDa E2 protein in the cells infected with AcCA327 (data not shown), suggesting that processing of HCV polyprotein occurred properly (Matsuura *et al.*, 1994). These results indicated that HCV structural proteins were expressed in mammalian cells by recombinant baculoviruses and processed properly.

Discussion

In this study we demonstrated that the baculovirus vector can deliver foreign genes into not only hepatocytes but also other mammalian cells such as HeLa, CPK and COS7 cells and high-level expression was achieved from the CAG promoter. In the previous studies, very low-level expression was observed in HeLa cells infected with the baculovirus with CMV promoter (Hofmann *et al.*, 1995). We demonstrated that HeLa cells infected with the baculovirus carrying the luciferase gene under the CAG promoter exhibited 10-fold higher luciferase activity than those infected with the virus driven by the CMV promoter. In addition, we demonstrated that the recombinant baculovirus is efficient enough to express the reporter gene in 90–100% of HepG2, Huh7, HeLa, CPK and COS7 cells. The CAG promoter used in this study is derived from the chicken β-actin promoter and can be utilized in a wide variety of cell lines (Niwa *et al.*, 1991; Fujita *et al.*, 1995; Kojima

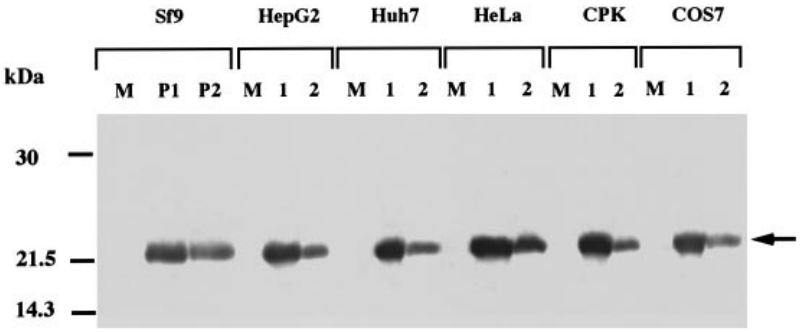

Fig. 7. Immunoblot analysis of the HCV core protein. Various mammalian cell lines were infected with the recombinant baculoviruses AcCA39 and AcCA327, respectively. As a positive control, Sf9 cells were infected with the recombinant baculovirus Ac39 and Ac327, respectively. Cells were harvested at 48 h after infection. As a negative control, extracts from mock-infected cells were examined. Cell extracts were separated by 12·5% SDS–PAGE and analysed by immunoblotting by use of mouse monoclonal antibody raised against the HCV core protein. M, mock-infected cells; P1, Sf9 cells infected with Ac39; P2, Sf9 cells infected with Ac327; 1, cells infected with AcCA39; 2, cells infected with AcCA327. Cell lines are indicated above. The numbers at the left represent molecular mass markers. The arrow indicates HCV core protein.

et al., 1995). These results suggest that foreign gene expression by baculovirus vectors is promoter dependent. By using a ubiquitously strong promoter it may be possible to establish a versatile expression system which is functional in various cell lines. Alternatively, it may be also possible to establish a selective expression system by a specific promoter that can work only in targeted cells.

AcNPV is considered to enter mammalian cells via an endosomal pathway (Hofmann *et al*., 1995; Boyce & Bucher, 1996). Expression levels of the *lacZ* gene increased in a dose-dependent manner according to the m.o.i. of the recombinant baculovirus. Furthermore, baculovirus was shown to infect various mammalian cells, suggesting that the virus utilizes a common molecule as a receptor on the cells. The exact mechanism for the entry of the baculovirus into mammalian cells is not known. Identification of a baculovirus receptor is essential to understand the entry mechanism of the virus into mammalian cells.

Viral vectors, generally very useful for study of the expression of foreign genes *in vitro* and *in vivo*, sometimes cause complex effects originating from their own replication or gene expression. Vaccinia virus vector has been used for expression of foreign genes because of its high expression in many cell lines. However, severe CPE caused by replication of vaccinia virus interfere with a long-term analysis of the expressed products (Wyatt *et al*., 1995). Expression of certain viral genes induces cytotoxicity and stimulates virus-specific cellular immune response eventually leading to destruction of the host cells. This is noted even when replication-defective adenovirus vectors were used for gene transfer (Yang *et al*., 1994). We observed extensive CPE in cells infected with defective adenoviruses at a high m.o.i. On the other hand, no visible CPE were detected in the cell lines inoculated with the baculovirus even at a high m.o.i. and they grew normally. In this study, the titres of infectious virus started decreasing immediately after infection and no infectious virus was recovered at 9 days after inoculation. It is noteworthy that luciferase activity was continually detected in cells infected with the recombinant baculovirus carrying the luciferase gene for 14 days after inoculation. Mittal *et al*. (1993) reported that the half-life of luciferase is approximately 6–8 h in infected cells. Our results indicate continual synthesis of mRNA and long-term expression with the baculovirus system. Further study will be needed to determine whether intrinsic viral genes are expressed in mammalian cells or not.

In order to assess the usefulness of the baculovirus vector system, we constructed recombinant baculovirus containing the HCV genome as an example. HCV structural proteins were expressed at high levels. Furthermore, the expressed protein was post-translationally processed properly. Although some lymphocyte cell lines have been shown to support the replication of HCV (Shimizu *et al*., 1992), there is no cell culture system yet efficient enough to be used for detailed virological study. Hepatocytes are the major, if not only, sites of HCV replication (Negro *et al*., 1992). We have constructed recombinant baculoviruses for expression of various regions of the HCV genome. Studies of pathogenesis and replication of HCV are now in progress.

Although further study will be required before application for gene therapy, our results, in conjunction with the properties of the recombinant baculovirus – for example, large capacity of incorporation of foreign genes and easy selection of recombinant virus (Luckow & Summers, 1988) – suggest that the baculovirus vector is a good tool for gene delivery into various mammalian cells.

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