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The human polyomavirus, JCV, does not share receptor specificity with SV40 on human glial cells

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The initial event in the life cycle of a virus is its interaction with specific receptors present on the surface of a cell. Understanding these interactions is important to our understanding of viral tropism and tissue specific pathology associated with viral disease. The human polyomavirus, JCV, is the etiological agent of the fatal central nervous system (CNS) demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML is the direct result of JCV infection of oligodendrocytes, the myelin producing cell in the CNS. *In vivo*, JCV can be detected in oligodendrocytes, astrocytes, lymphoid tissue, and peripheral blood of PML patients. *In vitro*, JCV infects human glial cells, tonsilar stromal cells, and, to a limited extent, human B lymphocytes. The initial step in infection of cells by JCV is at the level of attachment and entry. A specific cell surface receptor for JCV on human glial cells has not been identified. To begin to understand the nature of JCV receptors on human glial cells, large quantities of a previously characterized hybrid JC virus (Mad-1/SVEΔ) were purified. A direct virus binding assay demonstrated that these highly purified and labeled JCV virions bound to a finite number of cellular receptors on human glial cells. A competitive virus binding assay demonstrated that an excess of unlabeled JCV competed with labeled JCV more efficiently than did an excess of purified SV40. Furthermore, anti-class I antibodies which inhibited infection of glial cells by SV40 had no significant effect on infection by JCV. These results imply that JCV does not share receptor specificity with the related polyomavirus, SV40.

Keywords: JCV; PML; polyomavirus; receptors; virus SV40;

Introduction

Seroepidemiological studies indicate that greater than 70% of the human population worldwide is infected with the human polyomavirus, JCV (Major *et al*., 1992; Walker and Frisque, 1986). The mode of virus transmission is unknown, and no clinical illness has been associated with primary infection. Like other polyomaviruses, infection with JCV is associated with the establishment of lifelong latent or persistent infections (Norkin, 1982; Walker and Frisque, 1986). Reactivation of JCV in chronically immunosuppressed patients leads to virus dissemination to the central nervous system and subsequent infection of glial cells (Lipton, 1991; Major *et al*., 1992; Telenti *et al*., 1992; Tornatore *et al*., 1994). Alternatively, JCV genomes may establish latency in the CNS and become reactivated during immunosuppression (Elsner and Dorries, 1992; Mori *et al*., 1991; Quinlivan *et al*., 1992; Vago *et al*., 1996; White *et al*., 1992). The primary targets of virus infection in the CNS are oligodendrocytes and astrocytes. The fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), results from lytic destruction of oligodendrocytes by JCV. JCV has also been associated with several neural and non-neural tumors, including medulloblastoma, neuroblastoma, glioblastoma, pineocytoma, and B cell lymphoma (Major *et al*., 1992; Tornatore *et al*., 1994). Recently, the JCV oncoprotein, large T antigen was detected in an oligoastrocytoma in an immunocompetent patient (Rencic *et al*., 1996).

The initial step in the establishment of virus infection is the interaction between the virus and receptors present on the surface of cells and tissues. In general, viruses that have a very narrow host range and tissue tropism, such as JCV, are often shown to interact with high affinity to a limited number of specific receptors present on susceptible cells (Haun *et al*., 1993; Marsh and Helenius, 1989). In some instances virus tropism is strictly determined by the presence of specific receptors that

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mediate binding and entry (Bai *et al*., 1993; Dalgleish *et al*., 1984; Haywood, 1994; Klatzman *et al*., 1984; Mendelsohn *et al*., 1989; Philipson *et al*., 1968; Racaniello, 1990; Tomassini *et al*., 1989; Weiss, 1993; Weiss and Tailor, 1995; Wickham *et al*., 1993). In other instances, however, successful entry into a cell is necessary but not sufficient for virus growth (Atwood, 1991; Bass and Greenberg, 1992; Mei and Wadell, 1995; Rossman, 1994). In these cases, additional permissive factors that interact with viral regulatory elements are required.

SV40 is the only member of the polyomavirus family for which a specific cell surface protein has been identified as a virus receptor (Atwood and Norkin, 1989; Breau *et al*., 1992). In a series of studies it was found that major histocompatibility complex (MHC) encoded class I proteins were a major component of the SV40 receptor on rhesus monkey kidney cells (Atwood and Norkin, 1989; Breau *et al*., 1992). It has recently been shown that entry of infectious SV40 into cells is mediated by caveolae and that MHC class I proteins play an essential role in targeting of SV40 to these structures (Anderson *et al*., 1996; Stang *et al*., 1997). The distribution and levels of MHC class I protein expression on numerous human cell types do not always correlate with susceptibility to infection by SV40 (Atwood, 1991; Basak *et al*., 1992). This indicates that additional factors contribute to SV40 tropism. These additional factors may be specific transcription factors, the presence of a co-receptor, or both. It is clear from several studies that the mouse polyomavirus (PyV) and the lymphotropic papovirus (LPV) do not share receptor specificity with SV40 (Clayson and Compans, 1989; Haun *et al*., 1993; Sanjuan *et al*., 1992). N-linked glycoproteins containing terminal α2-3 linked sialic acids are a critical component of the cell surface receptor for the mouse polyomavirus (Cahan and Paulson, 1980; Cahan *et al*., 1983; Fried *et al*., 1981; Hermann *et al*., 1997). Recent studies have suggested a possible role for α2-6 linked sialic acids as well (Chen and Benjamin, 1997). The cell surface receptor for the lymphotropic papovirus, LPV, involves O-linked glycoproteins with terminal α2-6 linked sialic acids (Huan *et al*., 1993; Keppler *et al*., 1994, 1995).

Very little is known about the early steps of JCV binding to and entry into glial cells. Studies addressing this critical point in the virus life cycle have been hampered by the relatively poor growth of JCV, even in human glial cells. We have overcome this problem by using a previously characterized hybrid JC virus (Mad-1/SVEΔ). The non-coding regulatory region of this virus is a hybrid between SV40 and the Mad-1 strain of JCV (Vacante *et al*., 1989). The coding sequences, including the late capsid coding region, are entirely from the Mad-1 strain of JCV (Vacante *et al*., 1989). When propagated in the human SV40 transformed glial cell line, SVG, Mad-1/SVEΔ yielded 30-fold higher levels of virus than the Mad-4 strain of JCV. We have taken advantage of this and successfully purified and labeled JCV virions derived from the Mad-1/SVEΔ infected glial cells. The binding of labeled JCV virions to SVG cells was saturable, indicating that JCV binds to a limited number of cell surface receptors. In a competitive virus binding assay, an excess of unlabeled JCV virions competed with labeled JCV virions more efficiently than did an excess of purified SV40 virions. Furthermore, pretreatment of glial cells with anti-MHC class I antibody inhibted infection by SV40 but not by JCV. These results imply that JCV does not share receptor specificity with the related polyomavirus, SV40.

Results

High level production of Mad-1/SVEΔ in SVG cells

We compared the growth of the Mad-1/SVEΔ hybrid virus with a Mad-4 isolate by infecting SVG cells with identical amounts of either virus. At weekly intervals the cultures were assessed for cytopathic effect and the supernatants collected and assayed for virus by hemagglutination assay (HA). At 1 week post-infection the Mad-1/SVEΔ infected cultures began to show signs of cytopathology when compared to uninfected controls (Figure 1). By 2 weeks post-infection significant cytopathology was observed in the Mad-1/SVEΔ infected cultures (Figure 1). By 3 weeks post-infection the Mad-1/SVEΔ infected SVG cells showed extensive cytopathology and were harvested for virus stock (Figure 1). Mad-4 infected cells did not show

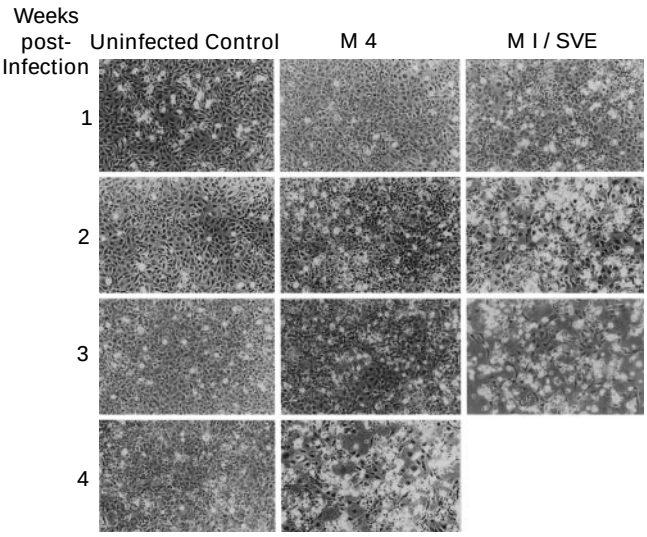

Figure 1 Glial cell cytopathology induced by infection with the Mad-4 (M4) and Mad-1/SVEΔ (M1/SVE) strains of JCV. SVG cells were either mock infected (uninfected control) or infected with equivalent doses of JCV strains are indicated. At weekly intervals the cells were monitored for cytopathic effect by phase contrast microscopy. Magnification=32×.

appreciable cytopathology until 3 weeks post-infection (Figure 1). At 4 weeks post-infection when the Mad-4 infected cultures showed extensive cytopathology the cells were harvested for virus stock (Figure 1). Weekly virus yields from the Mad-1/SVEΔ infected cells were always significantly higher than from the Mad-4 infected cells (Table 1). The total virus yield from the Mad-1/SVEΔ infected cultures was 32 times greater than the Mad-4 infected cultures (Table 1).

Determination of the purity of cesium chloride FITC-labeled JCV

Polyacrylamide gel electrophoresis and Western blotting were used to assess the purity of the FITC-labeled virus preparation. Five micrograms of FITC-labeled virus was resolved on a reducing and denaturing 12% polyacrylamide gel. A single FITC-labeled band migrating at approximately 39 kD was apparent upon exposure of the gel to UV light. This suggests that the FITC label is exclusively associated with the major JCV capsid protein, VP1 (Figure 2, lanes 7 and 8). The 39 kD band and some additional bands were seen after the gel was stained with Coomassie blue (Figure 2, lanes 5 and 6). A duplicate gel was blotted to a Nytran membrane and individual lanes on the blot probed with either anti-BSA or anti-SV40 antisera. The anti-SV40 antisera has been previously shown to cross-react with JCV VP1. The blots were then incubated with a secondary goat anti-rabbit antibody conjugated to horseradish peroxidase. Specific bands were detected by a chemiluminescence assay. Purified BSA and cesium chloride purified SV40 were used as controls for the anti-BSA antibody and the anti-SV40 antibody respectively. The anti-SV40 antibody confirmed that the 39 kD band seen in the gel exposed to UV light and in the Coomassie blue stained gel was indeed JCV VP1 (Figure 2 lane 4). We were able to detect BSA contamination in our cesium chloride purified FITC-labeled JCV preparation which is consistent with the recent published work of others (Figure 2 lane 3) (Frye *et al.*, 1997). The BSA contaminant in the purified virus preparation, however, was not significantly labeled by FITC (Figure 2, lanes 7 and 8).

JC virus binding to SVG cells

FITC-labeled virus binding to SVG cells was detected by incubating increasing concentrations of labeled virus with 2×10^5 SVG cells for 30 min on ice. FITC-labeled BSA diluted identically was used as a negative control for background fluorescence in these experiments. The cells were analyzed on a Becton-Dickinson FACScalibur™ flow cytometer. Data on the percentage of cells binding virus and the mean fluorescence intensity of virus binding was obtained. Dose dependent and saturable binding of FITC-labeled virus to SVG cells was observed (Figure 3). Saturation was observed with 1024 HAU of labeled JCV.

Table 1 Total virus yield measured by hemagglutination assay.

Weeks Post-infection	*Mad-4*	*Mad-1/SVE*
1	80	2560
2	7680	122 880
3	7680	409 600
4	12 800	ND

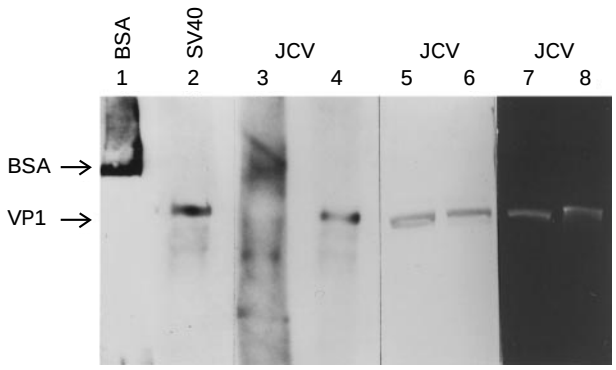

Figure 2 Western blot analysis of cesium chloride purified FITC-labeled JCV. Lane 1. Western blot of bovine serum albumin (BSA) detected with an anti-BSA antibody as a control. Lane 2. Western blot of cesium chloride purified SV40 detected with a polyclonal anti-SV40 antiserum as a control. Lane 3. Western blot of cesium chloride purified FITC-labeled JCV probed with an anti-BSA antibody to determine the level of BSA contamination of the virus preparation. Lane 4. Western blot of cesium chloride purified FITC-labeled JCV probed with a polyclonal anti-SV40 antiserum. Lanes 5 and 6. Coomassie blue stained gel of two separate preparations of cesium chloride purified FITC-labeled JCV. Lanes 7 and 8. Two preparations of FITC-labeled JCV exposed to UV light on an unstained gel.

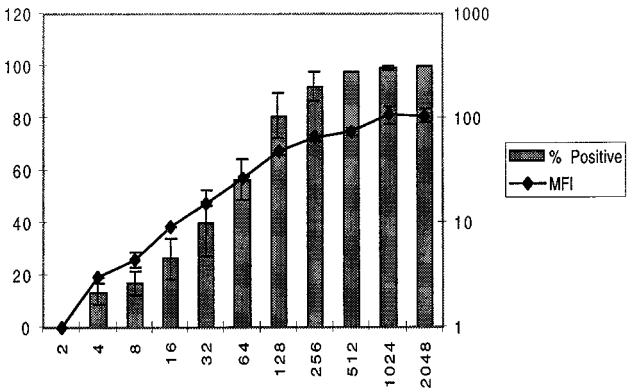

Figure 3 Flow cytometric analysis of FITC-labeled virus binding to SVG cells. Mean Fluorescence Intensity (MFI) is plotted on the right Y-axis and is indicated by a solid line. The percentage of cells binding virus is plotted on the left Y-axis and is indicated by solid bars. Virus dilutions corresponding to the hemagglutination titer (HAU) are shown on the X-axis.

Competition between JCV and SV40 for binding to SVG cells

To determine whether JCV and SV40 compete for binding to SVG cells both viruses were purified by cesium chloride density gradient centrifugation. The amount of protein in the purified virus samples was determined as described in Materials and methods. For the competition experiments SVG cells were incubated in buffer alone or in buffer containing a 100-fold or a tenfold excess of either unlabeled JCV, unlabeled SV40, or unlabeled BSA. FITC-labeled JCV was then added and the amount of labeled virus bound determined by flow cytometry. The binding of FITC-labeled JCV to SVG cells in the presence of a 100-fold and a tenfold excess of unlabeled JCV was reduced by 38% and 20% respectively (Figure 4a and c). In contrast, the binding of FITC-labeled JCV to SVG cells in the

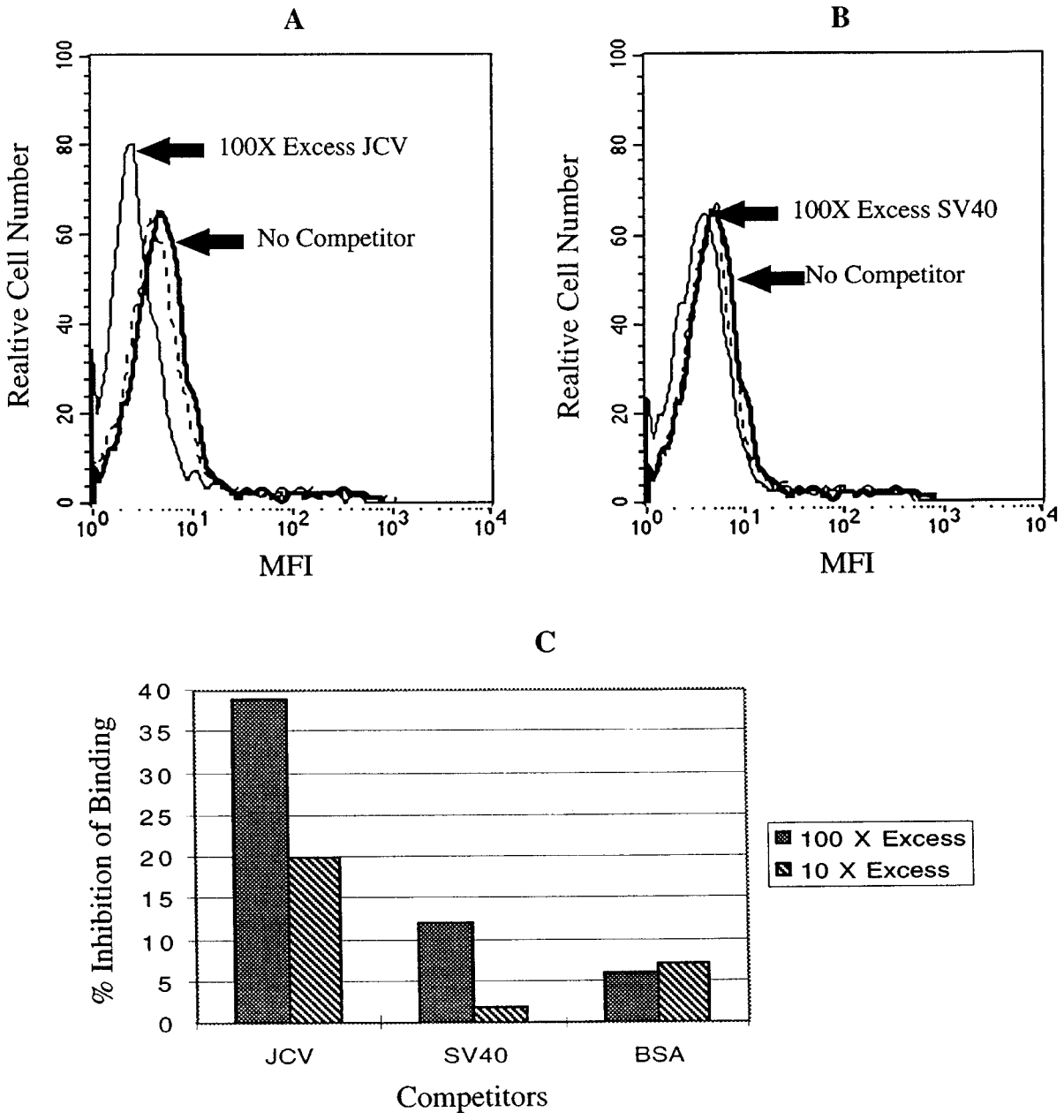

Figure 4 (**A** and **B**) Flow cytometric analysis of competition between JCV and SV40 for binding to SVG cells. FITC-labeled JCV was incubated with SVG cells in the presence and absence of either an excess of unlabeled JCV (**A** and **B**, thick solid line). JCV binding in the presence of a 100-fold excess of unlabeled JCV (**A**, thin solid line), or SV40 (**B**, thin solid line). JCV binding in the presence of a tenfold excess of JCV (**A**, dashed line) or SV40 (**B**, dashed line). (**C**) Graphic representation of the percent inhibition of binding by a 100-fold and a tenfold excess of unlabeled JCV, unlabeled SV40, or unlabeled BSA. MFI (Mean Fluorescence Intensity).

presence of a 100-fold and a tenfold excess of unlabeled SV40 was reduced by 12.8% and 3% respectively (Figure 4b and c). A 100-fold and a tenfold excess of BSA inhibited the binding of labeled JCV by 5.2% and 6% respectively (Figure 4c).

Effect of anti-class I antibody on infection of SVG cells by JCV and SV40

MHC class I proteins are known to be a component of the SV40 receptor on monkey kidney cells (Atwood and Norkin, 1989; Breau *et al*., 1992). The direct competition experiment above does not rule out the possibility that JCV also associates with MHC antigens on SVG cells. MHC class I proteins are not constitutively expressed on glial cells *in vivo*, however, under a variety of pathological conditions, MHC antigen expression can be induced on these cells. One report demonstrated the presence of MHC class I proteins on glial cells in active PML lesions (Achim and Wiley, 1992). This raises the possibility that JCV, like SV40, could potentially associate with MHC class I antigens on the cell surface. To address this possibility we first determined whether our glial cell line expressed MHC class I antigens. SVG cells were incubated with a dilution series of the monoclonal anti-MHC class I antibody, BB7.7. The cells were then washed 3 × and incubated with a secondary goat anti-mouse FITC labeled antibody. Constitutive expression of class I antigens was detected on these cells (Figure 5). We next asked whether this anti-class I antibody could inhibit infection of SVG cells by JCV. As a control we tested the ability of the antibody to inhibit infection of SVG cells by SV40. Pretreatment of the cells with anti-class I antibody inhibited infection by SV40 by 57% (Figure 6, compare the stippled bar to cross hatched bar). In contrast the same pretreatment had no significant effect on infection by JCV (Figure 6, compare the stippled bar to the cross hatched bar).

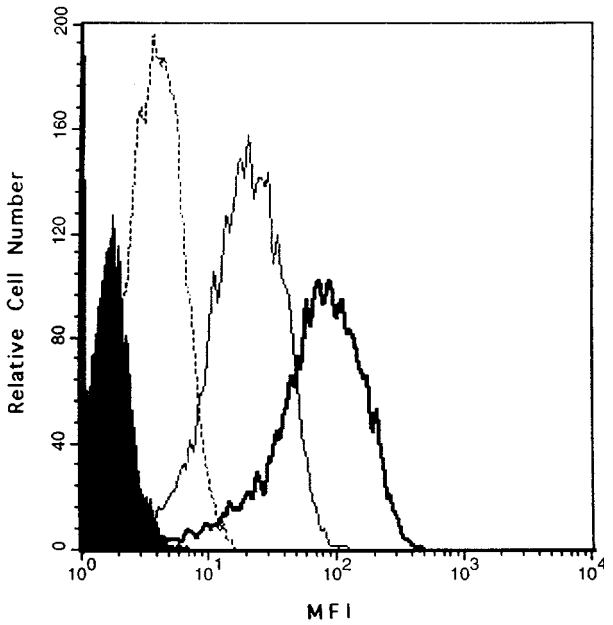

Figure 5 Flow cytometric analysis of MHC class I expression on SVG cells. SVG cells were incubated with anti-class I antibody undiluted (thick solid line), diluted 1 : 10 (thin solid line) or diluted 1 : 100 (dashed line) followed by addition of a secondary FITC-labeled antibody. The solid curve are SVG cells incubated with secondary antibody alone. The relative number of cells is plotted on the Y-axis and mean fluorescence intensity (MFI) is plotted on the X-axis.

Discussion

The results presented here represent the first attempt to study the interaction of JCV with cellular receptors on human glial cells. This critical event in the life cycle of JCV has not previously been studied due to the relatively poor growth of JCV, even in human glial cells. We have taken advantage of a previously characterized JC virus that contains a hybrid JCV/SV40 regulatory region. The hybrid virus induced significant cytopathology in the SVG human glial cell line and large numbers of progeny virions were produced. The receptor binding characteristics of the hybrid virus are those of the Mad-1 strain of JCV. The increased growth of the hybrid is due to the presence of a hybrid SV40/JCV regulatory region (Vacante *et al*., 1989). These attributes make this hybrid virus ideal for studying JCV receptor interactions.

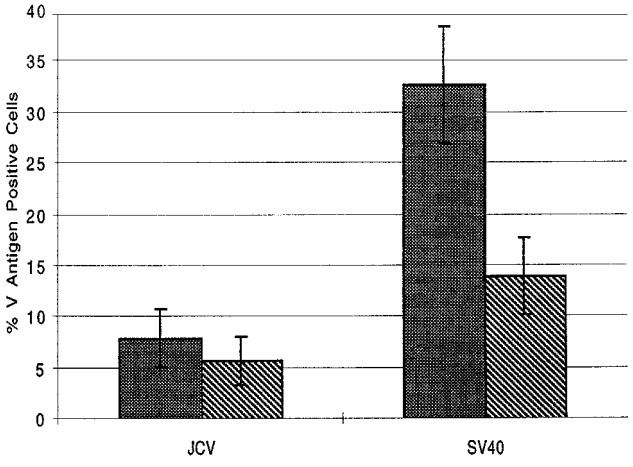

Figure 6 Infection inhibition assay. SVG cells were infected with SV40 or JCV in the presence (cross hatched bars) and absence (stippled bars) of anti-class I antibody. Infected cells were detected by indirect immunofluorescent assay of V antigen expressing cells and scored by counting. The percentage of V antigen positive cells is plotted on the Y-axis. Error bars were derived from the square root of the total number of events counted. On average 300 microscopic fields (40 ×) containing between 70–100 cells were counted for each sample.

To study the binding of JCV to glial cells we chose to label the virus with FITC. This labeling method was chosen so that virus binding to discreet populations of cells could be detected by flow cytometry. In our first virus binding experiment we observed that 1024 hemagglutination units of FITC-labeled JCV completely saturated cell surface receptors on 2×10^5 SVG cells. It has been estimated that one hemagglutination unit contains 1×10^7 physical particles (Crawford, 1969). Using this estimate we can calculate the number of JCV particles in 1024 hemagglutination units at 1×10^{10}. This indicates that an SVG cell contains at least 50 000 JCV receptors. Similar estimates have arrived at 90 000 SV40 receptors on monkey kidney cells, 25 000 polyomavirus receptors on mouse fibroblasts, and 800–1800 LPV receptors on human B cells (Clayson and Compans, 1988; Haun *et al*., 1993; Herrmann *et al*., 1995, 1997).

To determine the specificity of JCV binding to glial cells we measured binding in the presence and absence of an excess of unlabeled JCV, unlabeled SV40, or unlabeled BSA. We found that labeled JCV was competitively inhibited by an excess of unlabeled JCV but not by an excess of SV40 or BSA. The relatively low amount of inhibition even at a 100-fold excess of unlabeled JCV suggests that a significant portion of JCV binding to glial cells is non-specific. Experiments are underway that will directly measure the kinetics of JCV binding to glial cells.

As SV40 infection of monkey kidney cells is known to be inhibited by antibodies directed at MHC class I proteins we tested whether these antibodies would inhibit infection of glial cells by JCV. We first confirmed that MHC encoded class I proteins were expressed on SVG cells. We next tested the ability of anti-MHC antibody to inhibit infection of the SVG cells by both SV40 and JCV. We found that the anti-class I antibody only inhibited infection by SV40. This result indicates that infection of SVG cells by JCV is not dependent on an interaction with class I proteins. We have previously demonstrated that anti-class I antibodies have no effect on infection of primary cultures of human glial cells by the Mad-1 strain of JC virus (our unpublished observations). These results, when taken together, imply that JCV and SV40 do not share receptor specificity on human glial cells. The nature of specific JCV receptors on glial cells and their role in determining viral tropism is currently under investigation.

Materials and methods

Cells, virus and antibody

The human glial cell line, SVG, was derived by transformation of human fetal glial cells by an origin defective SV40 mutant and has been previously described (Major *et al*., 1985). SVG cells were maintained in a humidified 37°C CO_2 incubator in Eagles' Minimum Essential Media (E-MEM; Mediatech Inc., Herndon, VA) supplemented with 10% heat inactivated fetal bovine serum (Mediatech Inc., Herndon, VA). Hybridoma cells were grown in suspension in RPMI-1640 Hybrimax® media (Sigma, St. Louis, MO) supplemented with 10% heat inactivated fetal bovine serum (Mediatech Inc., Herndon, VA). The Mad-4 strain of JCV was isolated from cultures of persistently infected Owl monkey glioblastoma cells and has been previously described (Major *et al*., 1987). The hybrid Mad-1/SVEΔ virus was constructed by insertion of the regulatory region of SV40 into the regulatory region of the Mad-1 strain of JCV (Mad-1/SVE) (Vacante *et al*., 1989). Propagation of Mad-1/SVE in human glial cells led to deletions and alterations exclusively in the regulatory region. The rearranged regulatory region contains the origin of replication, the TATA box and 78 base pairs of the first 98 base pair repeat from JCV and one complete 72 base pair repeat from SV40. Most of the 72 base pair repeats and the 21 base pair repeats from SV40 were deleted. The virus is termed Mad-1/SVEΔ to indicate this fact. A comparison of the restriction patterns of Mad-1/SVEΔ DNA with the prototype Mad-1 DNA were identical except for the regulatory region changes just discussed (Vacante *et al*., 1989). No additional alterations were apparent following subsequent passage of Mad-1/SVEΔ in human fetal glial cells (Vacante *et al*., 1989). The host range of the hybrid has been extended to include human embryonic kidney, and Rhesus monkey fetal glial cells (Vacante *et al*., 1989). SV40, strain 776, was propagated in the African green monkey kidney cell line, CV1. CV1 cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and were maintained identically to SVG cells. The PAB597 hybridoma produces a monoclonal antibody against the SV40 major capsid protein, VP1 and was generously provided by Ed Harlow. This antibody has previously been shown to cross-react with JCV VP1 (Atwood *et al*., 1995). The BB7.7 hybridoma produces a monoclonal antibody directed at major histocompatibility encoded class I proteins and was obtained from ATCC.

Virus purification and labeling

For purification of Mad-1/SVEΔ virions, 1×10^8 SVG cells were incubated with 3200 hemagglutination units (HAU) of virus at 37°C for 1 h. Supernatants and dead cells were collected weekly from the infected cultures and stored at −20°C. At 3 week post-infection when the cells showed extensive cytopathic effect they were removed from the dishes by scraping. The dead cells and culture fluid from the previous 2 weeks were combined with this material and pelleted by centrifugation at 2000 r.p.m. for 30 min. The

resulting cell pellet was suspended in 30 ml of the supernatant and subjected to 3 freeze-thaw cycles. Deoxycholic acid was then added to 0.25% and the suspension was incubated at 37°C for 1 h. Cell debris was removed by centrifugation at 5000 r.p.m. and the supernatants were layered on a cushion of cesium chloride (CsCl) (1.34 g/ml). Virus was banded by centrifugation for 24 h at 35 000 r.p.m. in a SW55Ti rotor. The virus band was removed and dialyzed extensively against phosphate buffered saline (PBS) (137 mM NaCl, 2.682 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.2). Purified virus was stored in 100 μl aliquots at -80°C. Virus titers were determined by hemagglutination assay (HA) on human type O erythrocytes. SV40 was purified in an identical manner from infected CV1 cells. SV40 titers were determined by plaque assay. A portion of the gradient purified JCV was dialyzed against fluorescin isothiocyante (FITC) labeling buffer (0.05 M Boric Acid, 0.2 M NaCl, pH 9.2). The concentration of protein in the purified virus preparation was determined by the method of Bradford (1976). Purified virus (2.0 mg) was then incubated for 8 h at room temperature with 50 μl of a 5.0 mg/ml solution of FITC (Sigma, St. Louis, MO) dissolved in DMSO (Sigma, St. Louis, MO) (Harlow and Lane, 1988). The FITC-labeled virus was purified a second time by centrifugation over a cushion of cesium chloride. The FITC-labeled virus band was visualized with a hand-held UV light, removed, and dialyzed extensively against PBS (pH 7.2). The ratio of FITC to protein was determined by spectrophotometry.

Western blotting and polyacrylamide gel electrophoresis

Equivalent amounts (5.0 μg) of purified bovine serum albumin (BSA) (Fraction V, Fisher Biotech, Springfield, NJ), gradient purified SV40, or gradient purified and FITC-labeled JCV were diluted in Laemmli sample buffer (0.5 M Tris-HCl (pH 6.8) 10% (v/v glycerol, 10% (w/v) SDS, 0.1% (w/v) Bromphenol blue containing β-mercaptoethanol. The samples were heated at 95°C for 5 min and resolved on a 12% Tris-HCl polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA) in Tris-glycine buffer containing SDS. The gel was exposed and photographed on a UV lightbox to detect FITC-labeled bands. Total proteins on the gel were detected by staining with coomassie blue. A duplicate gel was electroblotted to Nytran membranes (Schleicher and Schuell, Keene, NH). The efficiency of protein transfer was determined by staining the blot with 0.1% (w/v) Ponceau S. Individual lanes were cut into strips and the strips blocked overnight in 1 × TBST (137 mM NaCl, 25 mM Tris, 2.7 mM KCl, 0.05% Tween 20) containing 5% non-fat dry mlik. The strips were incubated overnight with rabbit anti-BSA antibody (Sigma, St Louis, MO), or rabbit anti-SV40 antibody (gift from L Norkin) diluted 1 : 1000 in blocking buffer. The strips were washed 1 × in RIPA buffer (150 mM NaCl, 1.0% (v/v) NP-40, 0.05% (w/v) deoxycholate, 0.1% (w/v) SDS, 50 mM Tris (pH 8.0) and 2 × in TBST. The strips were incubated for 4 h with a goat anti-rabbit HRP conjugated antibody (1 : 1000 dilution). The strips were washed 1 × in RIPA buffer and 2 × in TBST. The strips were developed by incubation in equal parts of chemiluminescent reagents (ECL-kit, Amersham, Arlington Heights, III) and bands were detected by after exposure on Kodak XAR film (Rochester, NY).

Antibody and virus binding assays

For virus binding assays, SVG cells were removed from culture dishes by incubation with Versene (0.15 M NaCl, 0.002 M KCl, 0.006 M Na_2HPO_4 0.001 M KH_2PO_4, 0.001 M EDTA) and suspended at a concentration of 2×10^5 cells/ml in Hank's Buffered Saline Solution containing 2% heat inactivated fetal bovine serum (HBSS/2% sera) (Sigma, St Louis). The cells were incubated on ice with increasing concentrations of FITC-labeled JCV virions or with an equivalent amount of FITC-labeled BSA as a negative control. After a 30 min incubation the cells were washed 2 × in ice cold HBSS/2% sera, 1 × in ice cold PBS, and were fixed in 1.0 ml of ice cold PBS containing 1% paraformaldehyde. The cells were then analyzed on a Becton-Dickinson FACScalibur™ flow cytometer. For antibody binding, SVG cells were removed from culture dishes by a brief incubation in a Trypsin/EDTA solution (0.05% trypsin, 0.53 mM EDTA-4Na) (Mediatech Inc., Herndon, VA). The cells were then washed 3 × in HBSS 2% sera and suspended at a concentration of 1×10^6/ml in HBSS 2% sera. 1×10^6 cells were then incubated with a dilution series of hybridoma tissue culture supernatant containing the anti-class I antibody BB7.7 for 30 min on ice. Control cells were incubated with media alone. The cells were then washed 3 × in HBSS 2% sera and incubated with a 1 : 50 dilution of FITC labeled goat anti-mouse IgG (Jackson Immunoresearch Laboratories Inc., West Grove, PA) for an additional 30 min on ice. The cells were then washed 2 × in HBSS 2%, 1 × in PBS, and fixed in PBS containing 1% paraformaldehyde. The cells were analyzed as described above. For the competition assay, 1×10^5 SVG cells were preincubated for 30 min at 4°C with or without 0.3 mg of unlabeled JCV, unlabeled SV40, or unlabeled BSA. FITC-labeled JCV virions (0.03 mg or 0.003 mg) were then added to the cells and incubated for an additional 30 min on ice. The cells were washed 2 × in HBSS 2% sera, 1 × in PBS, and fixed in PBS containing 1% paraformaldehyde. The cells were analyzed as described above.

Infectivity assay

SVG cells growing on duplicate coverslips were either mock infected or infected with increasing dilutions of SV40 or JCV. At days 1–6 post-infection the cells were washed 3× in PBS and fixed in ice cold acetone. The percentage of infected cells were determined by indirect immunofluorescent assay of V antigen expressing cells. This was done by incubating the fixed cells with a 1 : 10 dilution of the anti-V antigen monoclonal antibody PAB 597 for 30 min at 37°C. The cells were then washed 3× in PBS and incubated with a 1 : 50 dilution of goat anti-mouse FITC labeled antibody for 30 min at 37°C. The cells were washed 1× with PBS, 1× with PBS containing 0.02% Evan's blue (counterstain), and 2× with PBS. V antigen positive cells were visualized on a Zeiss epifluorescent microscope and scored by counting. On average 300 fields containing between 70 and 100 cells per field were counted. V antigen expression peaked at 2 days post-infection with SV40 and at 3 days post-infection with JCV. For the infection blocking experiment 2×10^5 SVG cells growing on duplicate coverslips were pretreated with 100 μl of media or 100 μl of undiluted anti-MHC class I antibody (BB7.7-hybridoma supernatant) for 30 min on ice. The cells were then infected with SV40 (MOI=1) or with 10 HAU of JCV for an additional 30 min on ice. The cells were washed three times in PBS and incubated at 37°C in media. At various times post-infection the cells were washed 3× in PBS and fixed for 10 min in ice cold acetone. The percentage of infected cells was determined by indirect immunofluorescent assay of V antigen expressing cells as described above.

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