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ORIGINAL PAPER

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Characterization and application of polyclonal antibodies that specifically recognize JC virus large T antigen

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Abstract *Polyomavirus* JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy, a fatal demyelinating disease of the central nervous system. Similar to other polyomaviruses, such as simian vacuolating virus 40 (SV40) and BK virus (BKV), JCV is also associated with human tumours. The *Polyomavirus* early protein large T antigen (TAg) plays a crucial role in tumour pathogenesis. An antibody to SV40 TAg (PAb416), which cross-reacts with TAgs of both JCV and BKV, has been used widely for the detection of JCV and BKV TAgs. As a consequence, it is difficult to discriminate between the TAgs of SV40, BKV and JCV by immunohistochemical analyses. In the present study, we generated JCV TAg-specific polyclonal antibodies (JCT629 and JCT652) by immunization of New Zealand white rabbits with synthetic peptides reproducing the JCV TAg carboxyl-terminal region as immunogens. Immunoblotting analyses indicated that the new antibodies bind specifically to JCV TAg, and not to those of SV40 or BKV. We also demonstrated that these antibodies can be used for immunoprecipitation, immunocytochemical analyses and immunohistochemical staining of routinely processed specimens. In conclusion, the newly generated JCV-specific TAg antibodies may be useful both in the investigation of the pathophysiological function of JCV TAg and in discriminating between *Polyomavirus*-related clinical samples.

Keywords JC virus · Large T antigen · Carboxyl-terminal region · Polyclonal antibody

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Introduction

JC virus (JCV) belongs to the polyomaviruses and is the causative agent of progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (CNS) [34]. Accompanying the wide use of post-transplantation immunosuppressive therapy and the epidemic of acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) infection, the number of PML patients is increasing [13].

JCV consists of an icosahedral protein capsid containing a double-stranded circular DNA genome. The virus genome is composed of three elements—the transcriptional regulatory region, the early protein coding region and the late protein coding region [16]. Simian vacuolating virus 40 (SV40) and BK virus (BKV) also belong to the polyomaviruses and possess structural and functional characteristics similar to those of JCV. Large T antigen (TAg), which belongs to the viral early proteins, is multifunctional and contains several functional domains [37]. TAg is phosphorylated and binds to the viral replication origin where TAg promotes unwinding of the DNA double helix [9] and recruitment of cellular proteins. TAg binds to p53, which plays a pivotal role in the check point during the cell cycle and in the regulation of apoptosis and also interacts with pRb resulting in

the release of E2F from the E2F–pRb complex and the expression of $p14^{ARF}$ [7, 10, 25].

It has been reported that TAgs of SV40, BKV and JCV bind to p53 [3, 38]. TAgs of these viruses show a high degree (up to 70%) of amino acid sequence conservation [16], except in the C-terminal regions, which have unique sequences and contain the putative functional region, the "host range domain (HRD)" [36]. The roles of SV40, BKV and JCV TAgs in tumourigenesis have been reported with regard to their transforming activities in culture [35], oncogenic potential in laboratory animals [6, 8, 18] and association with clinical human tumours. JCV TAg protein was detected in human brain tumour tissues, including oligodendroglioma, anaplastic oligodendroglioma, pilocytic astrocytoma, astrocytoma, glioblastoma multiforme and ependymoma [11], as well as some gastrointestinal tumours, including colon and oesophageal cancer [12, 14]. In addition, BKV TAg was detected in primary and metastatic urothelial carcinomas [17]. Unlike JCV and BKV, it is widely accepted that the natural host for SV40 is the rhesus monkey and not humans. However, SV40-contaminated poliovirus vaccines prepared in rhesus monkey cells were used in the USA prior to 1963, and the association of SV40 TAg with human cancer was first reported by Soriano et al. [41] in malignant melanoma metastases in 1974. Subsequently, the association between SV40 infection and human tumours, such as mesothaelioma, brain tumours and non-Hodgkin's lymphoma [2, 46], and SV40 TAg was detected in choroid plexus carcinoma and renal cell carcinoma in Li-Fraumeni syndrome [26]. Thus, *Polyomavirus* TAgs have occasionally been detected in human tumour tissues. However, some previous studies failed to find evidence of a significant association of JCV with human tumours [1, 23, 29, 39, 47], and so the relation is still controversial.

Although the antibody to SV40 TAg (PAb416), which has been shown to cross-react with JCV and BKV TAgs, has been used widely for the detection of JCV and BKV TAgs [11, 17], it is difficult to distinguish between SV40, BKV and JCV TAgs by immunostaining. In the present study, we generated polyclonal antibodies that specifically recognize JCV TAg by immunization of New Zealand white rabbits using JCV TAg C-terminal region peptide as immunogens. These antibodies did not cross-react with SV40 or BKV TAgs and were applicable for immunoblotting, immunoprecipitation, immunocytochemistry and immunohistochemistry for the routinely processed specimens. These antibodies may be useful for the discrimination of *Polyomavirus*-related diseases and JCV research.

Materials and methods

Immunogens and immunization

The regions and sequences of two peptides comprising the 629–647 and 652–672 amino acid (a.a.) residues of JCV TAg, used as immunogens for generating polyclonal antibodies to JCV TAg, are shown in Fig. 1 and Table 1. The amino acid sequences of these antigens are summarized in Table 1. These peptides were cross-linked with keyhole limpet hemocyanin (KLH) as a carrier protein. A cysteine residue was added to the N terminus of the peptide comprising the 629–647 a.a. residues for conjugation with KLH. The emulsion of KLH-peptide and Freund's adjuvant were injected subcutaneously three times into the dorsal sites of each of two New Zealand white rabbits. The total amount of immunized peptide used for immunization was 0.1 mg (Invitrogen Life Technologies, Carlsbad, CA, USA).

Cell culture

The human embryonic kidney cell line, HEK293 (JCRB 9068), was provided by the Health Science Research Resource Bank (Osaka, Japan). The JCV persistently infected cell line, JCI, was described previously by Nukuzuma et al. [30]. Both cell lines were cultured under 5% CO_2 at 37°C in Dulbecco's minimum essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and antibiotics (penicillin and streptomycin) from Sigma (St. Louis, MO, USA).

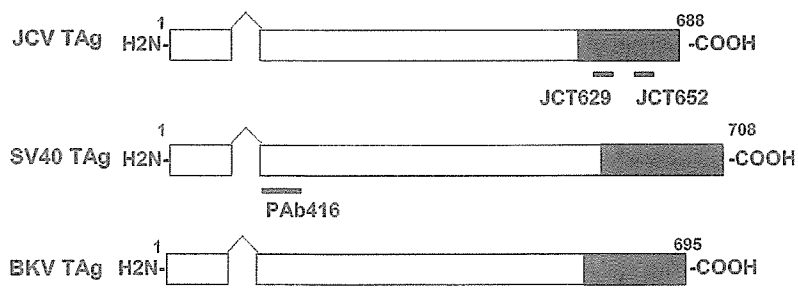

Fig. 1 Structure of the TAgs of JCV, SV40 and BKV and positions of the epitopes for the antibodies, including JCT629, JCT652 and PAb416. *Closed boxes* in the C-terminal regions of each TAg are relatively variable regions among these TAgs containing the host range domain (*HRD*). The epitope position of each antibody is indicated by a *bold line*. The numbers of amino acid residues are also indicated

Table 1 Amino acid sequence homology of TAgs of JCV, SV40 and BKV

	Position (JCV TAg)	JCV/SV40 (%)	JCV/BKV (%)
Overall	1–688	73	83
C terminus	621–688	47	65
Antibody	*Epitope*		
PAb416	82–129	73	86
JCT629	629–647	19	92
JCT652	652–672	22	37

Amino acid sequence homology between JCV and SV40 TAg or JCV and BKV is represented as percentages. Although the overall homology is up to 70% among the three TAgs, the homology of the C terminus is relatively low. The sequences and homology to TAgs of SV40 and BKV of synthetic peptides used for the generation of PAb416, JCT629 and JCT652 antibodies are shown
JCV JC virus, *SV40* Simian vacuolating virus 40, *BKV* BK virus, *TAg* large T antigen

Plasmid construction and transfection

Complementary DNAs of SV40, BKV and JCV TAgs were subcloned into the mammalian expression vector, pCXN2-Flag [44]. The introns of each TAg DNA were eliminated to abolish the production of small t antigen, which is also an early transcript of *Polyomavirus* [20]. Transfection into HEK293 was carried out using Lipofectamine 2000 reagent (Invitrogen Life Technologies) to HEK293 in accordance with the manufacturer's instructions. Briefly describing, the cells were subcultured on the day before transfection. For the 35 mm plates, the plasmids (2 μg) were diluted with 250 μl of Opti-Mem I, and Lipofectamine 2000 (5 μl) was diluted with 250 μl of Opti-Mem-I. After incubation for 5 min, these samples were combined and incubated for 20 min at room temperature. Thereafter, the mixed solution was added to the plates containing cells and antibiotic-free medium. At 48 h post-transfection, the cells were washed with phosphate-buffered saline (PBS) and subjected to immunoblotting, immunoprecipitation and immunocytological staining.

Antibodies

The antibody against SV40 TAg, Ab-2 (PAb416; Oncogene Science, Boston, MA, USA), was used as a control for immunoblotting, immunoprecipitation, immunocytological staining and immunohistological analyses. The newly generated polyclonal antibodies, whose immunogens consisted of the a.a. residues 629–647 and 652–672 of JCV TAg, were designated as JCT629 and JCT652, respectively. Anti-p53 and anti-actin monoclonal antibodies were purchased from DAKO (Glostrup, Denmark) and Chemicon (Temecula, CA, USA), respectively.

Immunoblotting

For immunoblotting analyses, TAg-transfected HEK293 cells were harvested 24–48 h after transfection, lysed in RIPA buffer [10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 50 mM NaF, 10% glycerol, 1% sodium deoxycholate, 0.1% SDS and 0.5 mM phenylmethylsulfonyl fluoride (PMSF)] and mixed with complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA) [32]. The cell lysates were fractionated by SDS-polyacrylamide gel electrophoresis, and the separated proteins were transferred onto polyvinylidene difluoride filters (Millipore, Bedford, MA, USA). The filters were incubated with primary antibodies overnight at 4°C. The dilutions of antibodies were as follows: PAb416 (1:1,000), JCT629 (1:1,000), JCT652 (1:1,000) and actin (1:1,000). After washing with TBS, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:3,000) at room temperature for 1 h. The immunopositive signals were detected using the ECL reagents (GE Healthcare Bio-Science Corp., Piscataway, NJ, USA) and analyzed with an LAS-1000 plus image analyzer (Fuji Film, Tokyo, Japan).

Immunoprecipitation assay

TAg-transfected HEK293 cells were lysed in binding buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.5, 1% Triton X-100) and incubated with antibodies and protein G-Sepharose beads (GE Healthcare Bio-Science Corp.) for 2 h at 4°C [33, 43]. Beads were pre-incubated in 1% BSA for 1 h to avoid non-specific direct binding of the proteins. After washing four times with binding buffer, the beads were boiled in sample buffer and the proteins were separated by SDS-PAGE. To avoid cross-reactivity of the secondary antibody with precipitated immunoglobulin heavy chain (55 kDa), detection of p53 (1:1,000) was performed using True Blot from eBioscience (San Diego, CA, USA).

Immunocytochemistry

Cells plated onto glass coverslips were fixed in 100% methanol at −20°C for 10 min. After blocking with 1% BSA for 30 min, cells were incubated with each antibody (1:500) overnight at 4°C [22]. The coverslips were washed five times with PBS, incubated with Alexa Fluor 488-conjugated anti-mouse or anti-rabbit immunoglobulin antibodies (1:500) at room temperature for 1 h and observed using a laser scanning confocal microscope (Olympus, Tokyo, Japan).

Immunohistochemistry

Consecutive sections of prostatic tumours expressing SV40 TAg of SV40 TAg transgenic rats [24] and BKV TAg-positive urinary tract sections [40] were used as positive controls. A total of six cases of PML positive for JCV capsid protein VP-1 immunostaining and four

non-PML brain samples from surgical and post-mortem samples were subjected to immunohistochemical analyses (Table 2). Formalin-fixed and paraffin-embedded sections were deparaffinized with xylene and rehydrated through a graded ethanol series. For antigen retrieval, sections were immersed in citrate buffer (pH 6.0) and heated using a pressure cooker. Thereafter, sections were treated with normal serum to eliminate non-specific binding of antibodies and incubated with 0.3% H_2O_2 methanol to quench endogenous peroxidase activity. After treatment, sections were incubated with primary antibodies, including PAb416 (1:500), purified JCT629 (1:10) and purified JCT652 (1:100), at 4°C overnight. After incubation with the biotinylated second antibody, immunoreaction products were visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB) [31]. Affinity purification of the new antibodies was performed as follows. The antiserum was diluted with one volume of borate-buffered saline (BBS) and passed through the peptide-conjugated columns. After washing out non-specific immunoglobulins and other proteins with BBS, the columns were eluted using BBS in a stepwise pH gradient (Invitrogen Life Technologies). We also performed double immunohistochemical staining to identify the cell types that were immunopositive for the newly generated antibodies. As markers of astrocytes and oligodendrocytes, anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (Nichirei, Tokyo, Japan) and anti-myelin proteolipid protein (PLP) monoclonal antibody (Sigma) were applied, respectively. After incubation with EnVison anti-mouse HRP second antibody (DAKO Cytomation, Carpinteria, CA, USA), immunopositive signals for GFAP or PLP were visualized with DAB. Thereafter, heat administration was performed for antigen retrieval and to quench extra HRP. The sections were incubated with anti-JCT antibody at 4°C overnight. After incubation with biotinylated second antibody and streptavidin alkaline phosphatase (AP), the signals were visualized with a Vector AP substrate kit (Vector Laboratories, Burlingame, CA, USA). As negative controls, non-diseased areas of non-PML brain samples were used.

In situ hybridization

In situ hybridization (ISH) with a JCV biotinylated DNA probe corresponding to the entire JCV genome (JC Virus BioProbe; Enzo-Diagnostics, Farmingdale, NY, USA) was performed using a capillary gap staining system (Fisher Biotech Micro Probe; Fisher Scientific, Pittsburgh, PA, USA). Paraffin sections 4 μm thick were deparaffinized and digested with pepsin in 0.1 N HCl. The probe was diluted in hybridization buffer (Enzo-Diagnostics) to a concentration of 1 μg/ml and incubated at 37°C for 3 h. After washing with 1× SSC and 0.2× SSC, the biotinylated probe was detected using a streptavidin AP detection system and Fast Red/naphthol phosphate (Farma Corporation, Tokyo, Japan) according to the manufacturer's instructions. After treatment, sections were counterstained with haematoxylin.

Results

JCT629 and JCT652 specifically recognized JCV TAg, but not SV40 or BKV TAgs

Immunoreactivity of the antibodies, including PAb416, JCT629 and JCT652, was estimated by immunoblotting (Fig. 2). PAb416 recognized SV40, BKV and JCV TAgs, and the intensities of each band were similar, suggesting that the sensitivity of PAb416 to each TAg was similar. Meanwhile, JCT629 and JCT652 specifically recognized JCV TAg, but did not cross-react with SV40 or BKV TAgs on immunoblotting (Fig. 2).

Application of JCT629 and JCT652 to immunoprecipitation assay to detect TAg binding protein

We next examined the possibility of using these antibodies for immunoprecipitation assay to investigate JCV TAg binding proteins. It has been reported that SV40, BKV and JCV TAgs bind to the tumour suppressor protein, p53. Using cell lysates from HEK293 cells expressing SV40, BKV and JCV TAgs, we investigated whether JCT629 and JCT652 have the

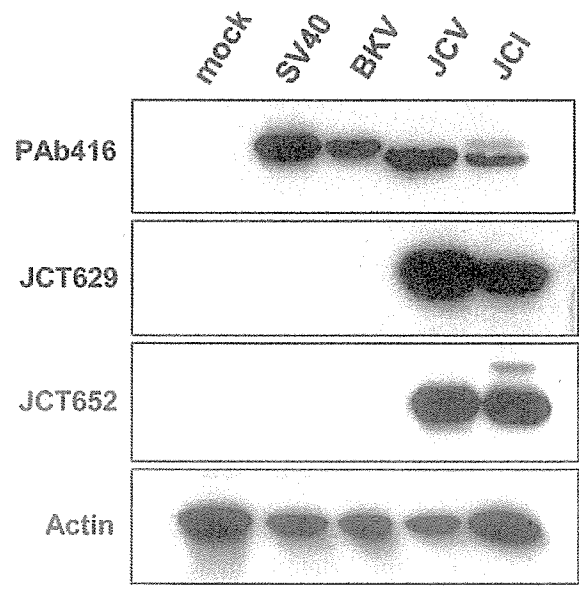

Fig. 2 Immunoblotting analyses with PAb416, JCT629 and JCT652 antibodies using SV40, BKV and JCV TAg-expressing HEK293 cells and JCV-producing cell line (*JCI*). PAb416 recognizes SV40, BKV and JCV TAgs (*top column*), while JCT629 and JCT652 specifically recognize recombinant JCV TAg and native JCV TAg, respectively, in JCI cells. Anti-actin antibody was used as an internal control (*bottom*)

potential to immunoprecipitate p53 and TAg complex. As shown in Fig. 3, PAb416 immunoprecipitated p53 bound with SV40, BKV and JCV TAgs, while JCT629 and JCT652 antibodies exclusively immunoprecipitated p53 bound with JCV TAg (Fig. 3). The precipitated immunoglobulin heavy chain (HC) was used as a control.

Application of JCT629 and JCT652 to immunocytochemical analyses

We also examined whether JCT629 and JCT652 antibodies were applicable to fluorescent immunocytological staining. PAb416 showed positive signals in the nuclei of HEK293 cells expressing SV40, BKV and JCV TAgs and in the persistently JCV-infected cell line, JCI (Fig. 4a–e). JCT629 and JCT652 antibodies exclusively recognized TAg in JCV TAg-expressing HEK293 cells (Fig. 4i, n) and JCI cells (Fig. 4j, o). The background staining levels of these new antibodies were slightly higher than that of PAb416.

JCT629 and JCT652 antibodies specifically recognized JCV TAg in tissue sections

To confirm the applicability of JCT629 and JCT652 antibodies to immunohistochemical analyses, we performed immunostaining of routinely processed paraffin-embedded tissues using these antibodies. For immunohistochemistry, the antibodies (JCT629 and JCT652) were purified using antigen affinity columns, because immune serum did not work in immunohistochemical staining. PAb416 showed positive signals in the nuclei of the prostate tumour cells from SV40 TAg transgenic rats (Fig. 5a) and urinary epithelia infected with BKV (Fig. 5d). The nuclei of glial cells of the PML brain, which sometimes contained a perinuclear halo, were also stained positive with PAb416 (Fig. 5g). Purified JCT629 and JCT652 showed positive signals exclusively in PML brain samples (Fig. 5h, i) and did not cross-react with cells expressing SV40 or BKV TAg (Fig. 5b, c, e, f). The staining patterns of JCT629 and JCT652, which showed diffuse staining in the nuclei, were similar to that of

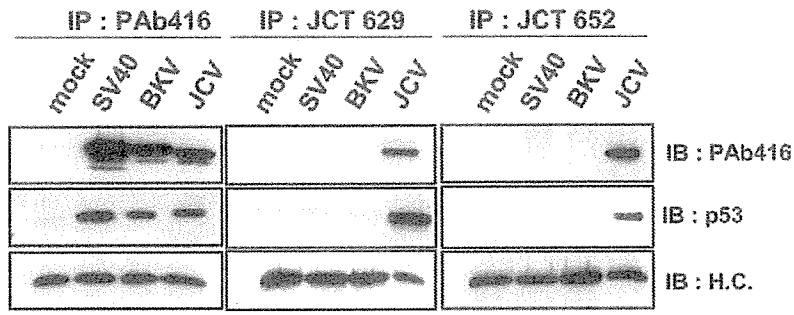

Fig. 3 Immunoprecipitation assay. The lysates from SV40, BKV and JCV TAg-expressing HEK293 cells were incubated with each antibody and protein G-Sepharose beads. The precipitates were subjected to immunoblotting with either PAb416 or anti-p53 antibody. PAb416 precipitated the complexes of p53 and each TAg (*top*). Meanwhile, the complex of JCV TAg and p53 was precipitated exclusively by JCT629 and JCT652. Precipitated immunoglobulins were recognized with horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin antibodies to confirm the amounts of precipitated antibodies. *H.C.* immunoglobulin heavy chain

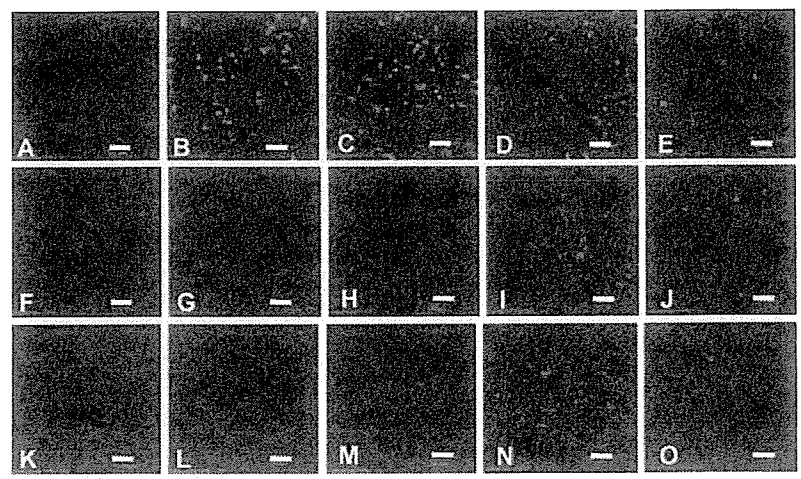

Fig. 4 Fluorescent immunocytological examination of SV40, BKV and JCV TAg-expressing HEK293 cells and JCI cells. PAb416 showed positive signals in the nuclei of cells expressing each TAg (**a–d**) and JCI cells (**e**). JCT629 (**f–j**) and JCT652 (**k–o**) interacted specifically with JCV TAg. Note that the latter two antibodies showed slightly higher background signals. *Bars* 100 μm

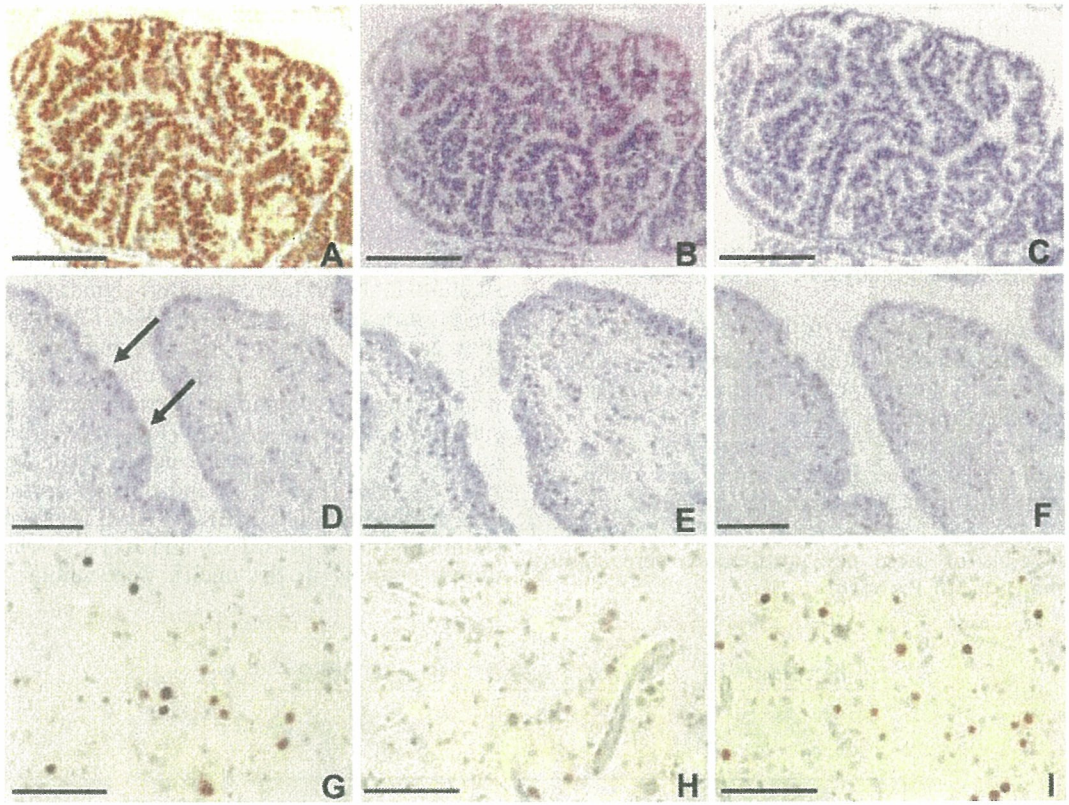

Fig. 5 Immunohistochemical analyses of each TAg-expressing tissue sample and PML brain. Prostatic tumour tissues from SV40 TAg-expressing transgenic rat (**a–c**), epithelia of the urinary tract, in which BKV had been demonstrated (**d–f**) and PML brain tissue (**g–i**) were subjected to immunohistochemical analyses. PAb416 (1:500 dilution) recognized all TAgs. Positive signals were detected in the nuclei of the prostatic tumour (**a**), the urinary epithelia indicated by the *arrows* (**d**) and cells with enlarged nuclei in the PML brain (**g**). In contrast, purified JCT629 (1:10) and JCT652 (1:100) specifically recognized JCV TAg (**h**, **i**) and did not cross-react with TAgs of SV40 (**b**, **c**) or BKV (**e**, **f**). *Bars* 100 μm (**a–c**, **g–i**), 50 μm (**d–f**)

PAb416; however, the sensitivity of the antibodies seemed less than those of PAb416. Two PML brain samples (patient numbers 2 and 4) were negative for JCT629 (Table 2).

Double immunohistochemical staining revealed that cells immunopositive with JCT652 antibody (blue colour) were negative for GFAP (brown colour in Fig. 6a, b) and positive for PLP (brown colour in Fig. 6c–e), suggesting that the immunopositive cells were oligodendrocytes. We also performed ISH for the detection of the JCV genome. All PML samples showed positive signals on ISH (Fig. 6f, g and Table 2), while non-diseased brain samples from non-PML cases were negative for JCT629, JCT652 and ISH.

Discussion

The anti-SV40 TAg antibody, PAb416, binds to SV40, BKV and JCV TAgs, because the epitope of PAb416 is a.a. 83–128 of SV40 TAg, which is a region highly conserved among SV40, BKV and JCV TAgs (Fig. 1 and Table 1). In the present study, we demonstrated that the newly generated polyclonal antibodies, JCT629 and JCT652, bind specifically to JCV TAg (Fig. 2), using JCV TAg C-terminal region peptides as immunogens. As JCT629 and JCT652 antibodies are applicable for biochemical assays, including immunoblotting and immunoprecipitation analyses (Figs. 2, 3), these antibodies are useful for the investigation of JCV TAg binding molecules.

SV40- and BKV-specific anti-TAg antibodies have been reported previously [19, 27], and these antibodies recognized the C-terminal regions of TAgs. Bollag and Frisque [4] reported that the monoclonal antibody, PAb2000, specifically recognized JCV TAg but not SV40 or BKV TAg. However, PAb2000 also recognized JCV small t antigen, which is produced by differential splicing of the viral early transcript and plays an important role in oncogenicity, because the epitope of PAb2000 is the N-terminal 81 a.a. of JCV TAg overlapping with the encoding region of JCV small t antigen. Although specific antibodies recognizing SV40, BKV and JCV TAgs have been reported, there have been no reports of immunohistochemical studies using these antibodies. Although the antibody,

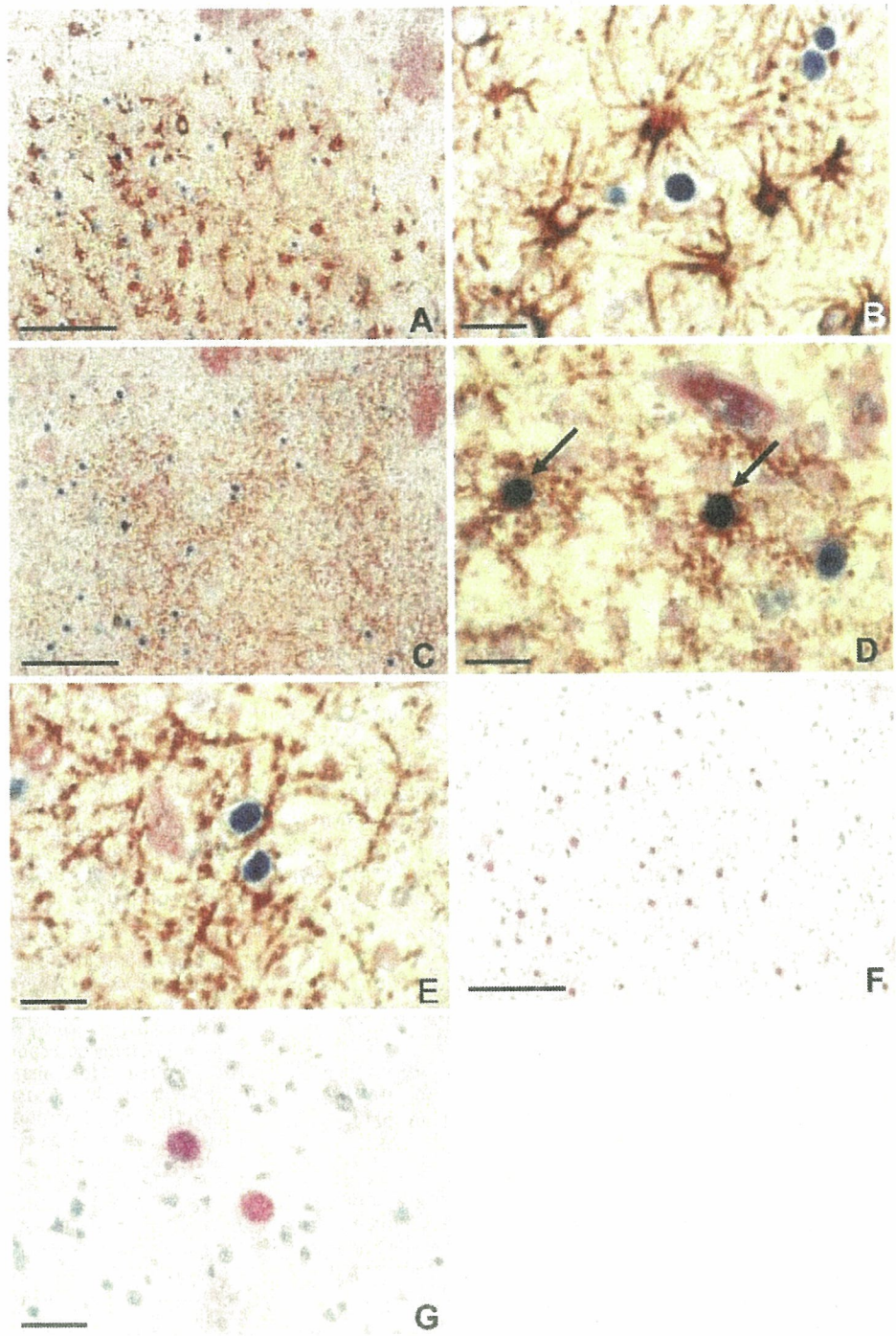

Fig. 6 Double immunohistochemical analyses and in situ hybridization (*ISH*) of PML brain tissue. Immunopositive signals of JCT652 and GFAP are represented as *blue* and *brown*, respectively (**a**). Higher magnifications are shown in (**b**). JCV TAg-positive cells were usually not co-localized with GFAP-positive cells. Meanwhile, JCV TAg-positive cells (*blue*) were co-localized with PLP (*brown*), indicated by the *arrows* in (**d**). The cells sometimes possessed perinuclear halo (**c–e**), suggesting that Tag-positive cells were oligodendrocytes. ISH analyses detected the JCV genome (*red*) in serial sections of the same tissue (**f**, **g**). *Bars* 200 μm (**a**, **c** and **f**), 50 μm (**b**, **d**, **e** and **g**)

PAb2003, established by Bollag et al. [5] was applicable to immunostaining of human brain tissue and has been reported to be a more powerful tool than ISH [28], PAb2003 cross-reacts with BKV TAg. Antibodies JCT629 and JCT652 can detect the TAg in the nuclei of the oligodendrocytes, which are the main target of JCV, by immunohistochemistry (Figs. 5, 6); however, the sensitivity of these antibodies was less than that of PAb416.

Recently, it was also reported that SV40 and BKV TAgs were detected in human tumour tissues [17, 26]. In addition, serological evidence of the exposure to SV40 in zoo workers [15] and detection of SV40 in the urine of children [45] suggested that SV40 infection may spread in the human population. Thus, using these new antibodies combined with either PAb416 or ISH, it may be possible to discriminate JCV TAg among the *Polyomavirus* TAgs related to human diseases.

Table 2 Profile of examined cases and immunohistochemical analyses

Patient no.	Age/sex	Material	Brain pathology	IHC				ISH
				VP-1	PAb416	JCT629	JCT652	
1	68/F	S	PML	+	+	+	+	+
2	49/F	S	PML	+	+	−	+	+
3	61/M	S	PML	+	+	+	+	+
4	50/M	P	PML	+	+	−	+	+
5	66/M	P	PML	+	+	+	+	+
6	36/M	P	PML	+	+	+	+	+
7	59/M	S	Non-PML (glioblastoma)	−	−	−	−	−
8	54/F	S	Non-PML (lung cancer)	−	−	−	−	−
9	37/M	P	Non-PML (drug-induced hyperthermia)	−	−	−	−	−
10	64/F	P	Non-PML (malignant melanoma)	−	−	−	−	−

In non-PML cases, brain samples without pathological finding were used for analyses
M male, *F* female, *S* surgical samples, *P* post-mortem samples, *IHC* immunohistochemistry, *ISH* in situ hybridization

Our results suggested that the C-terminal regions of JCV TAg used as immunogens may be located on the outer surface of the protein, and the antibodies could access and bind with the protein. Homology of the epitope of JCT629 (a.a. 629–647 of JCV TAg) with that of BKV is more than 90%, as shown in Table 1, and it was therefore unexpected that JCT629 did not cross-react with BKV TAg. One explanation is that conformational differences between these two proteins may be crucial for accessibility of the antibody. Further, these areas may contain specific functional domains in JCV TAg. It has been reported that the C-terminal region of SV40 TAg, including HRD, plays pivotal roles in the growth of adenovirus [21] and virion assembly [42]. Although the function of the C terminus of JCV TAg has not yet been precisely determined, it may be important in JCV infection.

Biochemical and immunohistological examination performed in the present study clearly showed that the newly generated antibodies, JCT629 and JCT652, specifically recognize JCV TAg. These observations provide a basis for the development of a useful immunological system for the detection and identification of JCV in clinical samples and for JCV research.

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