Identification of species-specific and cross-reactive epitopes in human polyomavirus capsids using monoclonal antibodies

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The human antibody response to polyomavirus capsid proteins is not well characterized. Recombinant BK virus (BKV), JC virus (JCV) and simian virus 40 (SV40) virus-like particles (VLP) were produced in a baculovirus system, and mouse monoclonal antibodies (mAbs) to these proteins were generated using standard methods. Nine of 12 BKV mAbs showed neutralizing activity. The non-neutralizing antibodies also bound BKV pseudocapsids in an ELISA binding assay. Most antibodies recognized conformational species-specific epitopes, but several exceptions were found: (i) BKV mAb BK-F11 cross-reacted with a linear buried epitope common to both JCV and SV40 pseudocapsids, (ii) two of six JCV antibodies (JC-6.7 and JC-7.9) and two of 13 SV40 antibodies (VP1-H2 and VP1-I2) recognized linear buried epitopes common to all three viruses and (iii) SV40 antibody VP1-E5 recognized a linear surface epitope on JCV pseudocapsids.

BK virus (BKV), JC virus (JCV) and simian virus 40 (SV40) belong to the genus Polyomaviridae. Polyomaviruses have been associated with several clinical syndromes. BKV is best known for causing the syndrome BKV nephropathy in kidney transplant recipients (Drachenberg et al., 1999, 2003; Drachenberg et al., 2001; Hirsch et al., 2001; Mylonakis et al., 2001; Nickelet et al., 1999; Ramos et al., 2002; Randhawa & Demetris, 2000; Randhawa et al., 1999, 2002a; Rosen et al., 1983; Vallbracht et al., 1993). JCV is associated with progressive multifocal encephalopathy in AIDS patients. SV40 has been sporadically isolated from human mesothelioma, lymphoma and brain tumours. The polyomavirus capsid consists primarily of viral capsid protein-1 (VP-1) which is directly involved in interactions with cellular receptors. Recombinant VP-1 produced in vitro readily assembles into virus-like particles (VLP) or pseudocapsids which resemble the native virion but lack the central core of viral DNA.

The antibody-mediated immune response to human polyomavirus capsid proteins requires further study. Serological studies show that most adults have had prior exposure to BKV. Marked elevations in anti-BKV antibody levels occur in patients with active viral replication (Bohl et al., 2005; Hamilton et al., 2000; Hariharan et al., 2005; Knowles et al., 2003; Randhawa et al., 2006). Antibodies to JCV are also widely prevalent in healthy subjects and in patients suffering from progressive multifocal encephalopathy. Antibodies to SV40 are reported to occur in only about 5% of healthy individuals and 16% of HIV-infected individuals (Jafar et al., 1998; Knowles et al., 2003). There is controversy over whether or not these antibodies are really the result of cross-reaction with BKV and JCV epitopes.

The nature of the antigenic epitopes that elicit antibodies to polyomavirus capsid proteins is largely unknown. Shah et al. (1977) used anti-SV40 sera raised in guinea pigs and rabbits to demonstrate a genus-specific antigenic determinant in the major capsid protein VP-1. This epitope was buried inside the viral particle and shared by BKV, mouse polyomavirus A2, rabbit vacuolating virus and stump tail macaque virus (Shah et al., 1977). Subsequently, other investigators showed that hyperimmune antisera raised against BKV did not neutralize SV40, while antisera to SV40 showed weak reactivity with BKV (Jafar et al., 1998). The present work undertakes a more detailed evaluation of the nature of BKV, JCV and SV40 epitopes using a panel of
monoclonal antibodies (mAbs) directed to VLPs produced by each of these three viruses.

BKV, JCV and SV40 VLPs were prepared as described previously (Carter et al., 2003). Hybridomas secreting mAbs reactive to VLPs were generated by using methods described by Christensen et al. (1996a, b) and Rizk et al. (2008) with some modifications. mAb reactivity against both intact and disrupted VLPs was assessed by virus-binding ELISA assays (Christensen et al., 1996a, b; Rizk et al., 2008). Antibodies reacting with intact but not disrupted pseudocapsids were interpreted as recognizing conformational epitopes located on the exposed surface of the viral capsid (surface epitopes). Persistent or residual reactivity after disruption was taken as evidence of linear epitopes. In case of reactivity to disrupted pseudocapsids only, epitopes were assumed not to be exposed on the surface (buried epitopes).

For virus neutralizing assays, mAbs were tested as tissue culture supernates diluted 1:100. A volume of 5 μl was incubated with 100,000 infectious BKV or SV40 virions at 37 °C for 2 h to neutralize VP-1 epitopes on the viral protein capsid. Antibody-treated and control virions were then used to infect WI-38 cells (BKV, ATCC no. VR 837) or CV-1 cells (SV40, stock no. 449). BKV neutralizing activity was assessed by quantifying viral load of cultures maintained in 25 cm² flasks on day 7. Technical details of this PCR assay have been published previously (Randhawa et al., 2002b). A >75% reduction in viral DNA yield compared with control cells was accepted as a criterion for antibody neutralizing activity. SV40 neutralizing activity was assessed by quantitative RT-PCR (qRT-PCR) analysis of Tag transcripts following infection of cells with mixtures of mAbs and SV40. A suspension of SV40 virions (a gift from M. J. Tevethia, Pennsylvania State University) was incubated with individual hybridoma supernatants (1:9, from M. J. Tevethia, Pennsylvania State University) was of mAbs and SV40. A suspension of SV40 virions (a gift of Tag transcripts following infection of cells with mixtures compared with control cells was accepted as a criterion for neutralization of the real-time assay. None of the JCV or SV40 antibodies tested at comparable protein concentrations (as judged by VLP binding ELISA assays) had any neutralizing effect, indicating that antibodies to BKV were recognizing epitopes that were not identical to those recognized by mAbs to JCV and SV40. This implies that BKV neutralizing epitopes are, in general, species specific and distinct from other epitopes that are more promiscuous in their antibody binding ability. However, BK-F11 showed binding to all three polyomavirus capsids.

Using SV40 VLP, mAbs VP1-A19, VP-L8 and VP1-S7 were designated as neutralizing based on >75% inhibition of large T-antigen signal relative to a control antibody JC 10.13, which showed no binding to SV40 VLP (Fig. 1b). For mAbs VP1-E5, -G3, -H2, -I2, -J4, -K8, -N7, -Q5, -3.6 and -9.6, the signal ratio using JC 10.13 as the denominator was close to 1.0. All non-neutralizing antibodies showed satisfactory binding activities to SV40 VLP. In binding assays there was only weak cross-binding to BKV (Fig. 2a) and JCV (Fig. 2b) VLP for some antibodies, while others were specific for SV40 (Fig. 2c).

Optical density (OD) values (at 410 nm) obtained from binding assays using BKV VLP with different mAbs are shown in Fig. 2(a). Mean OD₄₁₀ for BKV mAbs varied from 0.33 (BK-L5) to 2.56 (BK-Q2) with intact BKV pseudocapsids. Disrupted BKV pseudocapsids were non-reactive except with BK-F11 (mean OD₄₁₀ 0.58). The non-neutralizing anti-BKV antibodies BK-M17, BK-O2 and BK-Q2 also showed good binding (OD₄₁₀ 1.54, 1.79 and 2.56, respectively) to BKV VLP. Although anti-JCV antibodies showed no neutralizing activity against BKV VLP (Fig. 1a), evidence of binding to BKV pseudocapsids was generally demonstrable (Fig. 2a). JC-2.5, JC-3.6, and JC-9.16 bound to intact viral pseudocapsids (mean OD₄₁₀ 0.49, 0.13 and 0.24). In contrast, JC-6.7 and JC-7.9 showed binding to disrupted pseudocapsids (mean OD₄₁₀ 0.89 and 0.64) but not intact pseudocapsids (mean OD₄₁₀ 0.04 and 0.002), suggesting that there is a buried capsid epitope that is not accessible in the native VLP configuration. JC-10.13 showed no binding activity. Anti-SV40 antibodies showed poor binding to intact BKV VLP (mean OD₄₁₀ 0.00–0.075) but VP1-H2 and VP1-I2 bound denatured BKV VLP (mean OD₄₁₀ 0.43, 0.17) (Fig. 2a).

In binding assays using JCV VLP, JCV mAbs bound to JCV VLP as expected, with mean OD₄₁₀ ranging from 0.510 (JC-3.6) to 2.06 (JC-10.13) (Fig. 2b). JC-2.5, JC-3.6, JC-9.16 and JC-10.13 recognized only intact pseudocapsids, presumably at exposed conformational epitopes. JC-2.5, JC-3.6, JC-6.7, JC-7.9 and JC-9.16 also showed cross-binding to BKV pseudocapsids (Fig. 2a). JC-3.6 and JC-9.16 also cross-bound intact SV40 pseudocapsids (Fig. 2c). JC-6.7 and JC-7.9 recognized only denatured JCV, BKV and SV40 pseudocapsids, suggesting the existence of linear buried epitopes common to all three human polyomavirus species studied.
In binding assays using SV40 VLP, all SV40 antibodies with the exception of VP1-G3 bound to SV40 as expected, with mean OD$_{410}$ ranging from 0.11 (VP1-I2) to 2.53 (VP1-J4) (Fig. 2c). VP1-A19, VP1-J4, VP1-K8, VP1-L8, VP1-N7, VP1-Q5 and VP1-S7 recognized only intact SV40 pseudo-capsids representing putative exposed conformational epitopes. All of these antibodies showed no cross-reactivity to BKV or JCV pseudo-capsids. VP1-E5 bound both intact and denatured pseudo-capsids and showed cross-binding to JCV pseudo-capsids. VP1-H2 and VP1-I2 reacted only with denatured pseudo-capsids with cross-binding activity for both BKV and JCV pseudo-capsids.

Collectively, these data illustrate the existence of neutralizing epitopes on BKV and SV40 capsids. Generally, antibodies with neutralizing activity to BKV pseudo-capsids did not show any cross-reactivity with JCV or SV40. Denaturation of VLP suggested that an epitope(s) recognized by these mAbs was primarily conformational in nature, as has been shown for human papillomavirus (Fleury et al., 2008). There were exceptions to this general rule. Thus, BKV mAb BK-F11 cross-reacted with a linear buried epitope common to both JCV and SV40 pseudovirions. Two JCV (JC-6.7 and JC-7.9) and two SV40 (VP1-H2 and VP1-I2) antibodies recognized linear epitopes common to all three viruses. An SV40 antibody VP1-E5 recognized a linear epitope on JCV pseudo-capsids. Conformational epitopes are generally more efficient in inducing a potent antibody-mediated response than linear epitopes. Thus, it has been shown that papillomavirus capsomeres (pentameric subcapsid particles produced in Escherichia coli) elicit lower antibody titres than intact VLP. On the other hand, papillomavirus capsids were as efficient as intact VLP in evoking CD8$^+$ T cell responses (Thönes et al., 2008).

Most viral epitopes appeared to be located on the exposed surface of the pseudovirion and were recognized in both intact and denatured pseudo-capsids. However, BK-F11, JC-6.7 and JC-7.9 epitopes appeared to be buried deeper in the protein capsid and became accessible only after denaturation. Antibodies to these epitopes would presumably appear later in the course of clinical infection after
infectious virions start losing capsid proteins within the host cell.

BKV, JCV and SV40 show approximately 70% homology at the DNA level. Hence, it is possible that antibodies raised against one virus might react against the other viruses. Studies performed in the late 1970s noted that some hyperimmune sera raised using whole viral particles reacted only with homologous virus, while others showed low-level cross-reactivity against heterologous viruses. Our work clearly shows the occurrence of B cell epitopes that are (i) shared by BKV and JCV capsids, (ii) shared by JCV and SV40 capsids and (iii) common to all three polyomaviruses. Prior work has documented VP-1 and large T-antigen-derived T cell epitopes common to BKV, JCV or SV40 (Krymskaya et al., 2005; Tevethia & Schell, 2001). Despite this sharing of antigenic epitopes, it has been possible to develop diagnostic serology assays for BKV that show little cross-reactivity with the related JCV (Viscidi & Clayman, 2006).

A noteworthy finding in this study was the demonstration of neutralizing activity of several anti-BKV mAbs. Neutralizing antibodies to the BKV capsid were first described by Flaegstad et al. (1986) who noted that titres of these antibodies correlated well with titres of haemagglutination inhibition antibodies. Subsequently, Jafar et al. (1998) demonstrated BKV neutralizing antibodies in subjects with and without human immunodeficiency virus (HIV) infection. Inter-species cross-reaction patterns were not evaluated. There is only one prior report that describes JCV neutralizing activity in a polyclonal antiserum produced in rabbits (Atwood, 2001). The occurrence of JCV neutralizing antibodies in human samples has not been described to our knowledge. However, SV40 neutralizing antibodies have been noted to occur in a small proportion of human sera obtained from newborn children, healthy adults, aboriginal populations, HIV-infected individuals and laboratory workers exposed to monkeys or simian cell lines (Jafar et al., 1998; Viscidi &
Clayman, 2006). There is considerable evidence that serological assays for SV40 cross-react with other polyomavirus species (Carter et al., 2003; de Sanjose et al., 2003; Engels et al., 2004).

The mAbs described here should have important diagnostic and therapeutic applications. No effective anti-BKV drugs are currently available for clinical use. Hence, following validation in appropriate experimental studies, BKV neutralizing antibodies deserve evaluation as a potential method for treating kidney transplant patients with active BKV viremia. An analogous potential therapeutic application would be to clear JCV viremia in AIDS patients and reduce the risk of development of progressive multifocal encephalopathy.

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References


