Prevalence and stability of antibodies to the BK and JC polyomaviruses: a long-term longitudinal study of Australians

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Serology has been used to indicate past infection by the human polyomaviruses BK virus (BKV) and JC virus (JCV), because the site of primary infection is not established fully. Little is known about BKV and JCV antibody stability over time. We investigated BKV and JCV seroprevalence and antibody stability over time in an Australian population-based study. Serum was collected from 458 adults participating in a longitudinal skin cancer study in Queensland in 1992, 1993 and 1996, and 117 people had a fourth sample collected in 2003. Serum samples were analysed for BKV and JCV VP1 antibodies by multiplex detection using the Luminex platform. The seroprevalence for BKV and JCV over 4.5 years was 97 and 63 %, respectively. The BKV seroprevalence was 99 % in 25–60-year-olds, and 94 % in people older than 60 years. JCV seroprevalence was around 60 % in people younger than 50 years, 68 % in people 50–70 years of age and 64 % in people older than 70 years. BKV seroprevalence was very stable over 11 years, with 96 % of people staying seropositive and 2 % remaining seronegative. JCV antibody status over time was less stable; 57 % of participants remained seropositive and 31 % seronegative. The same proportion of people (4 % each) seroconverted, seroreverted or had fluctuating JCV antibody levels. These results confirm the previously believed stability of polyomavirus antibodies, with BKV antibodies being highly stable and JCV antibodies moderately so. Thus, a single measure can be used as a reasonable indicator of long-term antibody status in epidemiological studies aiming to understand associations between polyomaviruses and disease.

INTRODUCTION

The human polyomaviruses BK virus (BKV) and JC virus (JCV) are small, circular double-stranded DNA viruses that infect a large proportion of people worldwide. Even though BKV and JCV were discovered almost 40 years ago, many aspects of the epidemiology of these two viruses are still unknown (Gardner et al., 1971; Padgett et al., 1971). Primary infection with BKV and JCV is usually asymptomatic and is believed to be transmitted through an oral or respiratory route in early childhood (Monaco et al., 1998). Both BKV and JCV have the ability to transform cells in vitro and it is well-established that the viral large T antigen is essential for transformation (Gordon et al., 1998; Shivakumar & Das, 1996). The polyomaviruses may therefore be involved aetiologically in a wide range of different cancers, but this has not yet been proven (Abend et al., 2009; Maginnis & Atwood, 2009; Rollison et al., 2009; Weinreb et al., 2006).

Because the primary infection with these viruses is asymptomatic and the site of primary infection is not established fully, serology has been used as a measure of infection. Until the late 1990s, serology studies were based on the haemagglutination-inhibition method, but, from the beginning of 2000, most studies have used a virus-like particle (VLP) ELISA (Hamilton et al., 2000; Stolt et al., 2003). More recently, multiplex detection with glutathione S-transferase (GST)-tagged proteins using the Luminex platform has become more common (Carter et al., 2009; Kean et al., 2009; Kjaerheim et al., 2007).

Infection with BKV generally occurs at an earlier age than infection with JCV, and the reported prevalence in healthy adults is around 90 % for BKV and 50–70 % for JCV (Kean et al., 2009; Stolt et al., 2003). BKV infects young children all over the world and seroprevalence usually peaks (close to 100 %) by the age of 10 years, and then declines in older age groups. In contrast, JCV infection rates vary more between populations, and seroprevalence usually increases with age and continues to rise throughout life (Kean et al., 2009; Stolt et al., 2003).
Little is known about BKV and JCV antibody stability over time. To our knowledge, this paper describes for the first time a long-term longitudinal study of BKV and JCV seroprevalence in a randomly selected group of adults from the general community.

**RESULTS**

Serum samples were collected from 458 participants in 1992, 1993 and 1996 and, of these, 117 also had a fourth serum sample collected in 2003. The mean age at baseline of the participants in the total cohort was 51 years (range, 27–76 years) and 55 % were female. Those with a 2003 sample were somewhat younger at baseline (mean age, 49 years) and 43 % were female.

The overall seroprevalence for BKV was 97 % in 1992, 1993 and 1996, and 96 % in 2003 (Table 1). JCV seroprevalence was 62–63 % in 1992, 1993 and 1996, and 69 % in 2003 (Table 1). For the subpopulation of 117 participants with four samples, BKV seroprevalence was almost identical to that of the 458 patients with three samples, but it was slightly higher for JCV, at approximately 68 % at all four time points. The mean and 95 % confidence interval (CI) of the median fluorescence intensity (MFI) values for each year and virus are presented in Table 1. The MFI values for BKV and JCV were uncorrelated with each other; Spearman's maximum correlation coefficient was −0.07 and no coefficients were significantly different from 0. We found a significant decrease in MFI values for BKV over time: a mean of 401 MFI year−1 between 1992 and 1996 (P < 0.0001) and 134 MFI year−1 between 1992 and 2003 for those participants with four samples (P < 0.0001). The decrease in MFI values for JCV was only significant for the group of people that had all four samples (mean MFI decrease, 50; P = 0.01).

BKV seroprevalence remained essentially the same when the MFI value used as cut-off was altered, i.e. there was <1 % change in either direction when the cut-offs used were 300 or 500 MFI (instead of 400 MFI). JCV prevalence was somewhat more sensitive to changes in the cut-off value, with up to a 5.5 % change.

The age-specific seroprevalences for BKV and JCV from 1992 are presented in Fig. 1. The BKV seroprevalence was close to 100 % in people under 60 years, but was slightly lower (93–94 %) for people aged 60 years and above. Age-specific seroprevalence for JCV was just below 60 % up to the age of 50 years, almost 70 % in people aged 50–70 years, and 64 % in those aged over 70 years, although these differences were not statistically significant.

### Changes in BKV and JCV antibody status over time

With regard to BKV antibodies, among the 458 people in the total cohort, 96 % were stably seropositive and 2 % remained seronegative during the 4.5 year follow-up. Nobody in this group seroconverted, but two participants...
(0.5 %) seroreverted (one seroreversion in 1993 and one in 1996), and eight (2 %) had fluctuating antibody levels (Table 2). The participants that had a fourth sample collected in 2003 had very similar serostability to those with only three samples [111 (95 %) seropositive, four (3 %) seronegative, no seroconverters, one seroreversion and one with fluctuating antibody levels over the 11 year period].

JCV antibody status over the 4.5 year time period was less stable than that of BKV (Table 2). Whilst 260 (57 %) remained seropositive and 142 (31 %) seronegative, 20 (4 %) participants seroconverted, 20 (4 %) seroreverted and 16 (4 %) had fluctuating antibody levels. JCV serostability for 11 years in the subgroup of participants with a fourth sample was very similar.

No association between sex and BKV serostability was found. For JCV, women (14, 3.1 %), were 2.4 times more likely to seroconvert than men (6, 1.3 %) and three times more likely to serorevert [15 women (3.3 %), five men (1.1 %)] (P=0.051). We found no significant associations between any other variables examined (age, skin type, occupational sun exposure, smoking or a diagnosis of skin cancer) and either BKV or JCV serostability.

Despite the relative stability of both BKV and JCV antibodies based on a cut-off to define positivity, there was marked fluctuation in the MFI values. In the 440 people who were stably seropositive for BKV, the mean difference in the MFI value (highest−lowest over the 4.5 year period) was 3051 (95 % CI, 2859–3243) and the mean ratio (highest/lowest) was 1.75 (95 % CI, 1.67–1.83). In 5 % of people, the MFI value only underwent an increase over time (mean ratio, 2.0; 95 % CI, 1.5–2.5), whilst in 37 % of people, the MFI value only decreased (mean ratio, 1.8; 95 % CI, 1.6–1.9). In 58 % of people, the MFI value fluctuated over the three measurement points (mean ratio, 1.8; 95 % CI, 1.7–1.9). Due to the small numbers, we have not presented increase or decrease of the MFI results for people who were stably seronegative or who changed their status.

For JCV, the mean difference between the highest and the lowest MFI among the 260 people who were stably seropositive was 2690 (95 % CI, 2366–3014) with a ratio of 1.7 (95 % CI, 1.6–1.8). Of these 260, 13 % had an increasing MFI value (mean ratio, 4.4; 95 % CI, 1.4–7.4), 24 % had a decreasing MFI value (mean ratio, 1.8; 95 % CI, 1.6–2.0) and 63 % had fluctuating MFI values (mean ratio, 2.6; 95 % CI, 1.5–3.8). The mean MFI ratio for the group of 20 seroconverters was 17.9 (95 % CI, 7.9–28.0), for 20 seroreverters it was 2.1 (95 % CI, 1.8–2.4) and for the 16 people whose serostatus fluctuated it was 3.8 (95 % CI, 2.2–5.4).

**DISCUSSION**

The overall seroprevalences reported here for both BKV and JCV are similar to those reported previously for healthy adult populations (Kean et al., 2009; Stolt et al., 2003) and were relatively robust to changes in the MFI cut-off used to determine positivity. Others have shown that BKV seroprevalence is acquired early in life, peaks in young children (90 %) and then drops off with increasing age to 70–80 % in people aged over 40 or 50 years, whereas JCV seroprevalence usually increases throughout life (Kean et al., 2009; Knowles et al., 2003). Whilst our age-specific JCV serology findings seem to be consistent with previous studies, a large proportion of our study population (aged between 25 and 60 years) had a BKV seroprevalence close to 100 % and we observed only a small decline in the older age groups. Our observation that there is no difference in seroprevalence with respect to sex has been described in previous studies (Abend et al., 2009; Kean et al., 2009) with the exception of one, which reported a significantly higher JCV seroprevalence in English men compared with women (Knowles et al., 2003).

Over time, we found the BKV seroprevalence to remain very stable. Almost all of the participants that tested seropositive or seronegative in the first sample retained this status over time. Nobody seroconverted and only a small proportion of people seroreverted or had fluctuating seropositivity. The JCV seroprevalence was less stable, with 88 % remaining stably seropositive or seronegative over 4.5 years and 81 % over 11 years. With the exception of an association between sex and JCV stability, we found no association between any variables examined and antibody stability.

There is limited information about BKV and JCV antibody within-person stability over time with which to compare our findings. To our knowledge, there are only two longitudinal serological studies on BKV and JCV, one in immunosuppressed patients (Randhawa et al., 2008) and the other in pregnant women (the Finnish Maternity Study; Stolt et al., 2003). Randhawa et al. (2008) investigated BKV antibodies in samples from 107 renal transplant recipients (RTRs) over a 1 year period with

| Table 2. Serostability for BKV and JCV in samples collected at three time points over 4.5 years (1992, 1993, 1996: n=458) |
|---|---|---|---|---|
| _Polyomavirus_ | _Serostability [% (n)]_ |  |
|  | Stable seropositive | Stable seronegative | Seroconversion | Seroreversion | Fluctuating |
| BKV | 96.1 (440) | 1.7 (8) | 0 | 0.5 (2) | 1.7 (8) |
| JCV | 56.8 (260) | 31.0 (142) | 4.4 (20) | 4.4 (20) | 3.4 (16) |
VLP-based ELISA. At the start of the study, 16 RTRs were BKV-seronegative, and 14 of them seroconverted during the following year. Seventeen of the 91 RTRs who were seropositive at baseline seroconverted (Randhawa et al., 2008). These patients showed large changes in seroprevalence and serostability over time, possibly due to disease or their immunosuppressive therapy after transplantation. The Finnish Maternity Study also used a VLP-based ELISA to analyse sera from 150 women aged 14–31 years who had a first serum sample taken during their first pregnancy and a second during their second pregnancy, with a follow-up period of up to 5 years (Stolt et al., 2003). Unlike our study, where we found some changes particularly in JCV serostability, none of these women changed their antibody status between the first and second sampling times. Differences may be due to the younger age of the Finnish study population and/or to differences in the method used for antibody detection.

We observed large variations in MFI values over time, even in those whose antibody status did not change over time. There was no overall upwards or downwards trend in the different serostability groups, with the majority of people having fluctuating MFI values. However, we found a significant decrease in MFI values year 

1, especially for BKV. The different types of variation in MFI values observed may be due to physiological variation, although the Finnish Maternity Study found that JCV and BKV antibody levels were close to identical in serial samples taken up to 5 years apart (Stolt et al., 2003). It is more likely that sample storage or other methodological issues and longer follow-up time are responsible for the variability that we observed in the present study. Despite the variation, we found that the actual seroprevalence was relatively robust to changes in the MFI value used to determine seropositivity.

Multiplex serology was initially developed for human papillomavirus (HPV) and has quickly expanded to also detect polyomavirus agents. There is limited information about how a multiplex analysis compares with two single-plex assays. However, the potential of HPV type-specific antibody detection by multiplex serology has been shown previously (Waterboer et al., 2005).

Using a uniform, arbitrarily defined cut-off to convert the continuous MFI measure into a binary variable is a limitation but, as long as there are no sera that can be used as international standards, any cut-off definition has an arbitrary component to it. However, whilst seroprevalence from different laboratories should be considered with caution, the primary focus of this analysis was on change in antibody status over time, so our results are internally valid irrespective of what cut-off is used. Furthermore, we found the seroprevalence to be very robust to changes in the cut-off used.

It is possible that antibody cross-reactivity for BKV and JCV VP1 antibodies was present. This can be only controlled experimentally by serum pre-incubation experiments, which are very laborious and therefore not applicable to large-scale seroepidemiological studies (Kjaerheim et al., 2007). However, even without serum pre-incubation, we did not find any correlation between BKV and JCV VP1 antibody reactivities. We therefore consider the antibody reactivities to be mainly virus-specific.

Our findings are likely to be broadly representative of JCV and BKV antibody prevalence and stability in Australian adults. The participants in the Nambour Skin Cancer Study have been shown to be similar to the population from which they were drawn (Green et al., 1999), and we did not find any demographic or behavioural differences between the 458 people included in this study and the remainder of the original cohort (n=1163) who did not have three serum samples collected and analysed.

These results confirm the previously believed stability of polyomavirus antibodies over up to 11 years in a randomly selected population of Australian adults. Thus, a single measure can be used as a reasonable indicator of long-term antibody status in epidemiological studies aiming to understand associations between polyomaviruses, especially BKV, and disease.

METHODS

Study population. This study was nested within the Nambour Skin Cancer Study, which has been described in detail previously (Green et al., 1999). Briefly, in 1992, 1621 randomly selected members of the Nambour Township in south-east Queensland, Australia, were enrolled in a trial of sunscreen and β-carotene for the prevention of skin cancer. All participants underwent an interview in which a detailed dietary, medical and occupational history was recorded. At least one serum sample was collected from 1398 participants in 1992, 1993, 1996 or 2003. Serum samples were collected from 693 randomly selected participants in 1992, 549 in 1993, 1211 in 1996 and 261 in 2003. In this study, we analysed data for 458 people who had blood collected in 1992, 1993 and 1996. Of these, 117 also had a fourth serum sample collected in 2003. The subgroup of 458 people and the 117 people with samples from 2003 studied here had similar characteristics to the remainder of the Nambour study population [mean age, 50 years (range, 26–76 years) and 55 % female]. This study was approved by the Ethics Committee at the Queensland Institute for Medical Research and all participants gave written informed consent.

Serology analysis. Serum samples were stored at −80°C and shipped on dry ice to DKFZ, Heidelberg, Germany, for serological analysis. The samples were analysed for antibodies to VP1 of BKV and JCV. The antibody-detection method was based on GST-capture ELISA (Sehr et al., 2001) in combination with fluorescent bead technology (Waterboer et al., 2005). Briefly, full-length viral proteins were expressed in bacteria in fusion with an N-terminal GST domain. Glutathione cross-linked to cascin was coupled to fluorescence-labelled polystyrene beads (SeroMap; Luminex), and GST-fusion proteins were affinity-purified on the beads directly in a one-step procedure. Bead types of different colour and each carrying a different antigen were mixed and incubated with human sera at a 1:100 dilution. Antibody bound to the beads via the viral antigens was stained by biotinylated anti-human Ig and streptavidin–R-phycocerythrin. A Luminex xMAP analyser was used to identify the internal colour of the individual beads and to quantify their reporter
fluorescence (expressed as MFI of at least 100 beads per set per serum). The serum samples were analysed on two consecutive days, and a quality-control (QC) panel of 188 sera was included to determine inter-day variation between samples analysed. The correlation for the raw MFI values for the individual 188 QC sera analysed on two consecutive days ranged from 0.83 to 1.00 (median, 0.96; Pearson’s correlation coefficient).

Statistical analysis. In the study, we included all participants who had polyomavirus antibody data available from sera collected in 1992, 1993 and 1996. A subgroup of these also had data for 2003 and we conducted additional analyses for this group.

Cut-off points for antibody positivity were selected by visual inspection of the distribution of MFI values among all study participants (Carter et al., 2009), and seropositivity was defined as having an MFI value of 400 or more. Associations with baseline positivity were analysed by using chi-squared tests. To analyse antibody stability over time, we classified participants as being seropositive at all time points (stably seropositive), seronegative at all time points (stably seronegative), seroconverting (change from seronegative to seropositive over time), seroreverting (change from seropositive to seronegative) or fluctuating between seropositive and seronegative (fluctuating). We used chi-squared tests to analyse associations between variables such as age, sex, skin type, sun exposure, skin cancer and antibody prevalence and stability. Differences and ratios in MFI over time were described by using simple descriptive statistics.

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REFERENCES


