SEROLOGICAL DIAGNOSIS OF HUMAN POLYOMAVIRUS INFECTION

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ABSTRACT

Measurement of antibody titres to the human polyomaviruses BK and JC has for many years had to rely on Haemagglutination inhibition. In recent years, viral serology based on virus-like particles (VLPs) in enzyme immunoassays (EIAs) has become widely used for a variety of viruses. We sought to establish a modern method for serological diagnosis of BK and JC viruses, by using purified yeast-expressed virus like particles (VLP) containing the VP1 major capsid proteins. Antibody titres in assays based on VLPs of BKV (strain SB) showed no correlation to the titres in similar JCV assays. BKV (SB) seropositivity increases rapidly with increasing age of the children and reaches a 98 % seroprevalence at 7–9 years of age, whereas JCV seroprevalences increase more slowly with increasing age reaching 51% positivity among children 9-11 years of age. The antibody levels are almost identical in serial samples taken up to 5 years apart, suggesting that both BKV and JCV VLP seropositivitities are usually stable over time and can be used to measure cumulative exposure to these viruses. Serology using SV40 VLPs showed strong cross-reactivity with human polyomaviruses, in particular with BKV strain AS, and establishing a specific VLP-based serology assay for SV40 required blocking with several hyperimmune sera to the human polyomaviruses. SV40-specific seropositivity also increased with increasing age of children, reaching 14% seroprevalence among children 7-9 years of age, but had limited stability over time in serial samples.

KEY WORDS: Human polyomaviruses**,** BKV strains, Simian virus 40, Seropositivity, Antibody stability, Haemagglutination inhibition (HI), Enzyme immunoassay (EIA), Virus Like particles (VLPs), Expression systems, *Saccharomyces cerevisiae***.**

INTRODUCTION

Different serological methods have been used over the years to measure antibodies to the Polyomaviruses. Haemagglutination inhibition (HI) assay has been the standard method for this purpose because of the ease and rapidity with which it could be performed[1].

Neutralization test and plaque reduction assay, where cell culture is required as neutralization of virus infectivity has also been described for SV 40 and BKV antibody detection[2, 3].

In recent years enzyme immunoassays (EIAs) has become widely used. A modern EIA based on virus-like particles (VLPs) has been established to assess polyomavirus antibodies in serum samples[4]. The Polyoma VLPs are based on the major capsid protein, VP1 and produced in yeast cells from *Saccharomyces cerevisiae*[5, 6].

Four antigenic variants of BKV have been described: the BKV prototype, BKV AS, BKV SB and BKV IV[7]. These BKV strains were isolated from urine specimens from several patient groups[8]. Each strain has been characterized by nucleotide sequencing of the VP1 region, which encodes the major capsid protein of BKV. Specific variations correlate with serological typing by Haemagglutination inhibition.

Infection with BKV occurs at an earlier age than does JCV infection. In the United States antibodies to BKV are acquired by 50 % of the children by the age of 3-4 years, whereas antibodies to JCV are acquired by 50 % of the children by the age of 10-14 years. The antibody prevalence to BKV reaches nearly 100 % by the age of 10-11 years and then declines to around 70-80 % in older age groups. The antibody prevalence to JCV reaches a peak of about 75 % by adult age[9].

In a recent study, serum samples from 290 children and 150 pregnant women stratified by age of first pregnancy were analysed for antibodies to polyomaviruses in a VLP-based antibody assay. Samples from 290 Swedish children aged 1–13 years, stratified in age groups with 2 year intervals demonstrated that BKV seropositivity increased rapidly with increasing age of

the children, reaching 98 % seroprevalence at 7–9 years of age, followed by a minor decrease[4, 10].

JCV seroprevalence increased only slowly with increasing age and reached a 51 % positivity among children 9-11 years of age[4] (Fig 1).

SIMIAN VIRUS 40

Antibodies against SV40 have been reported to be present in about 5% of healthy individuals from the US and India. Most reactivities are low-titered, but occasionally humans with neutralizing antibody titers of very high magnitude are found (similar titers as in experimentally infected monkeys). In the few and limited surveys that have been performed, there has been no correlation of SV40 seroprevalences with history of poliovirus vaccination. This has been interpreted as showing that SV40 is now circulating in human populations[11].

It is possible that the antibodies reacting with SV40 are induced by the human polyomaviruses and cross react with SV40. It is suggested that SV40 antibodies are cross-reactive BK antibodies and significant correlations have been reported between SV40 and BKV antibody levels, as well as with JCV antibody levels[4, 12]. In our studies SV40 antibodies were most closely related to BKV strain AS antibodies (correlation coefficient= 0.70)[4]. Sensitive and specific reagents for SV40 immune responses are needed to establish exposure to SV40 infection[13]. Only after blocking SV40 VLPs with high-titered hyperimmune sera against both BKV and JCV were we able to establish an SV40 serological assay devoid of cross-reactivity with BKV and JCV (unpublished observations). The SV40 seroprevalences increased with age of the children in a similar manner, as does BKV, with the peak being reached at 7-9 years of age with a 14% seroprevalence. SV40-specific antibodies appear to be less stable over time than BKV and JCV antibodies (unpublished observations).

ANTIBODY STABILITY

Polyomavirus seroprevalences appear to persist over time, but decrease slightly after early adulthood[15, 16]. Similar seroprevalences could either reflect that antibody levels are generally stable on the individual level or may reflect that antibodies both come and go at similar rates. In our recent study 300 serum samples were taken from 150 women during their first and second pregnancy[4]. The sera were obtained from the population-based serologic screening program for congenital infections at the first trimester of pregnancy[17, 18]. The 150 women were stratified by age of first pregnancy and had a second pregnancy during a 5-year follow up period. In the study, 50 women each were between 14-19 years, 20-25 years and 26-31 years of age at their first pregnancy[4].

The BKV and JCV seropositivities were almost identical in both samples in the 5-year follow-up study. The correlation between the antibody titres in the first and second pregnancies was very high: the correlation coefficient was 0.93 for SB and 0.94 for JC (Fig. 2a, b)[4].For both BKV SB and JCV, there were no cases of seroconversion on follow-up and no cases of seroreversion (loss of seropositivity), suggesting low acquisition rates of these infections in adulthood.

SEROLOGICAL METHODS

Haemagglutination inhibition (HI) has been the standard method for measurement of antibody titres to BKV and JCV. HI assays were used for this purpose because of the ease and rapidity with which they could be performed[1]. A lack of red blood cells agglutination means that antibodies are present and bind to viral antigen[19]. Both JC virus and BK virus have the ability to agglutinate human type O erythrocytes, unlike SV40. The major capsid protein VP1 is the predominant structural protein of the icosahedral virion particle and is responsible for attachment to cells and for erythrocyte agglutination[20].

Many contemporary assays for measuring antibodies to viral and other antigens employ enzyme immunoassay (EIA) techniques because of their greater sensitivity and precision relative to HI. Detection of JCV and BK virus (BKV) antigen in urine by antigen capture EIA was reported over a decade ago[21]. However, EIA for antigen capture or antibody detection did not become widely used because of the restricted range of cell types infectable by JCV, its lengthy growth cycle and poor replication capacity that made antigen preparation for use in EIAs labor intensive, time-consuming, and costly for testing large numbers of samples[1].

A comparison of antibody titers to JC virus (JCV) or BK virus (BKV) was made by haemagglutination inhibition (HI) and enzyme immunoassay (EIA) with 114 human plasma samples. JCV was grown in SVG cells, a cell line established by immortalization of human fetal brain cells with an origin-defective mutant of SV40. BKV (Gardner strain) was grown in low-passage human embryonic kidney (HEK) cells.

The viruses were purified by density gradient ultracentrifugation and used in EIA. Antibody titers to JCV or BKV determined by HI were lower than those determined by EIA. Nevertheless, as HI titers increased so did EIA titers. When antibody data were compared by the Spearman rank correlation test, highly significant correlations were found between HI and EIA titers. The results, in agreement with those of others, suggest that humans infected by JCV or BKV produce antibodies to species-specific epitopes on their VP1 capsid protein, which is associated with haemagglutination and cellular binding[1].

Plaque reduction assay using green monkey TC-7 cells has been described to detect SV40 serological reactivity. The plaque method is used for measuring viral infectivity and multiplication in cultured cells and whether antibodies have been able to neutralise this infectivity. Clear lysed areas or plaques develop as the viral particles are released from the infected cells[3].

Examining presence of cytopathic effect (CPE) has also been used in neutralisation assays for both BKV and SV40[2, 3].

In recent years viral serology based on virus-like particles (VLPs) in enzyme immunoassays (EIAs) has become widely used in several viral systems[22], including polyomavirus infections[4]. The polyoma EIA was based on yeast-expressed VLPs, containing the VP1 major capsid proteins of JC virus (JCV) and the AS and SB strains of BK virus (BKV)[23].

EIA SEROLOGICAL METHOD: The optimal concentration of polyomavirus VLPs and the serum dilutions used are determined by titration using positive and negative controls. Patients with PCR-detected BKV viruria usually have very high BKV antibody titres and serum samples from such patients are suitable as positive control reference samples. Samples from children of about 1 year of age rarely contain polyomavirus antibodies and are suitable negative control samples. We have used purified VLPs at a concentration of 6.25 ng per EIA well, coated in ice-cold PBS (pH 7.2). Half-area Costar 3690 EIA plates were incubated overnight at 4°C. After washing with 0.1% PBS/Tween, a blocking buffer consisting of 10% horse serum in PBS (HS-PBS) was added and incubated for 1 h at room temperature. The serum samples were diluted 1:40 in HS-PBS and incubated for 2 h at room temperature. The plates were washed five times with 150 ml PBS/Tween and an anti-human IgG mouse monoclonal antibody (Eurodiagnostica) diluted 1:800 was added and incubated for 90 min at room temperature. The plates were washed five times with 150 ul PBS/Tween and peroxidase-conjugated goat anti-mouse IgG (Southern Biotechnology) diluted 1:2000 in HS-PBS was added and incubated at room temperature for 60 min. Following another washing step, the peroxidase substrate ABTS was added and incubated for 30 min at room temperature and the absorbance measured at 415 nm[4].

PREPARATION OF VIRUS LIKE PARTICLES

Polyoma VLPs are based on the major capsid protein, VP1. The VP1 coding sequences were incorporated into the yeast expression vector pFX7 under the control of the galactose-inducible promoter. The pFX7- derived expression plasmids carrying the VP1 genes were transformed into the yeast *Saccharomyces cerevisiae* for cultivation. Polyoma VLPs from JCV, from the two antigenic variants of BKV (strains AS and SB) and from SV40 were produced in *S. cerevisiae* cells. Yeast transformation, cultivation and recombinant protein purification were carried out as described previously[5, 6]. Following disruption of yeast cells, lysate was centrifuged at 3000 g for 10 min at 4°C. Supernatants were then loaded on to a chilled 30% sucrose cushion and ultracentrifuged at 100 000 g for 3 h at 4°C. Resulting pellets were resuspended in 4 ml chilled disruption buffer and loaded on to CsCl gradients ranging from 1.23 to 1.38 g ml21 and centrifuged at 100 000 g for 48 h. Fractions of 1 ml were collected and subjected to SDS-PAGE analysis. Fractions containing protein corresponding to a molecular mass of ~40–45 kDa were pooled, diluted with 1.31 g CsCl ml21 and recentrifuged on a second CsCl gradient. Fractions were again collected and those containing VP1 were pooled and dialysed against PBS and analysed by SDS-PAGE, Western blot, electron microscopy (Figure 3) and HI[4, 23].

EXPRESSION SYSTEMS

The expression of recombinant VP1 with spontaneous assembly into virus-like particles (VLPs) has been demonstrated for a number of polyomaviruses using both prokaryotic and baculovirus systems. VLPs of JCV have also been expressed in *Escherichia coli* and polyomavirus VP1 will also assemble into capsid-like particles in the nucleus of insect cells when expressed in the baculovirus system[24, 25]. Yeast-derived recombinant VLPs offer many

advantages over other expression systems in terms of protein yield, cost and ease of protein expression[5, 23].

The VLP-based assays are likely to be useful in determining past exposure to BKV and JCV in epidemiological studies and for serological diagnosis in patients at risk of polyomavirus-associated diseases or showing early signs of complications due to polyomavirus reactivation[4].

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FIGURES

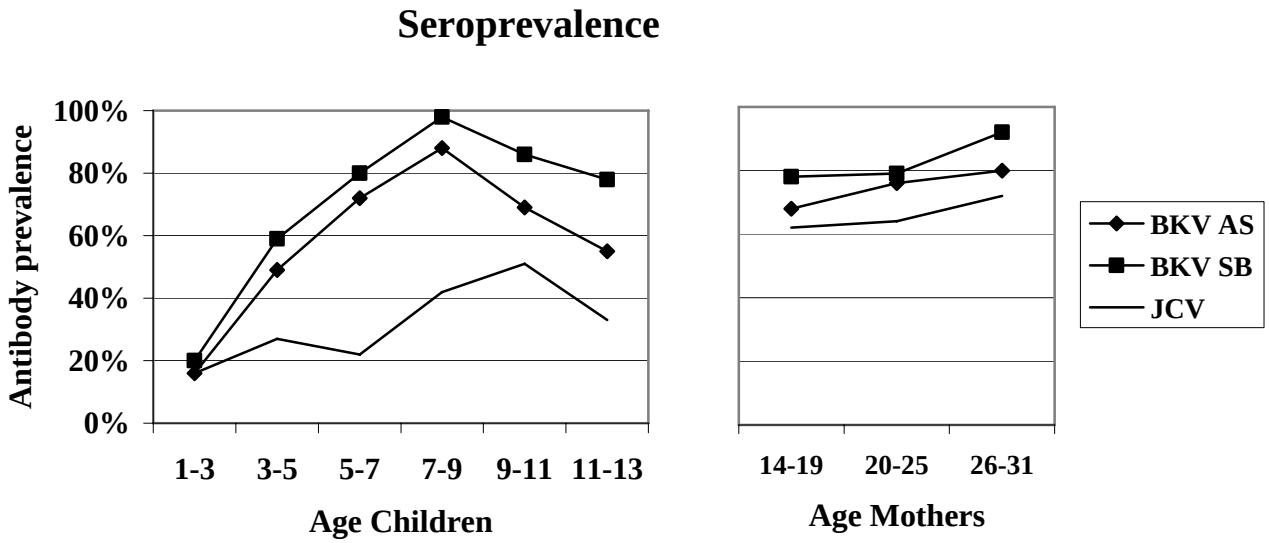

Fig 1. Prevalence of antibodies to the polyomaviruses BKV SB, BKV AS and JCV in children and pregnant women. Modification of a graph in reference[4]

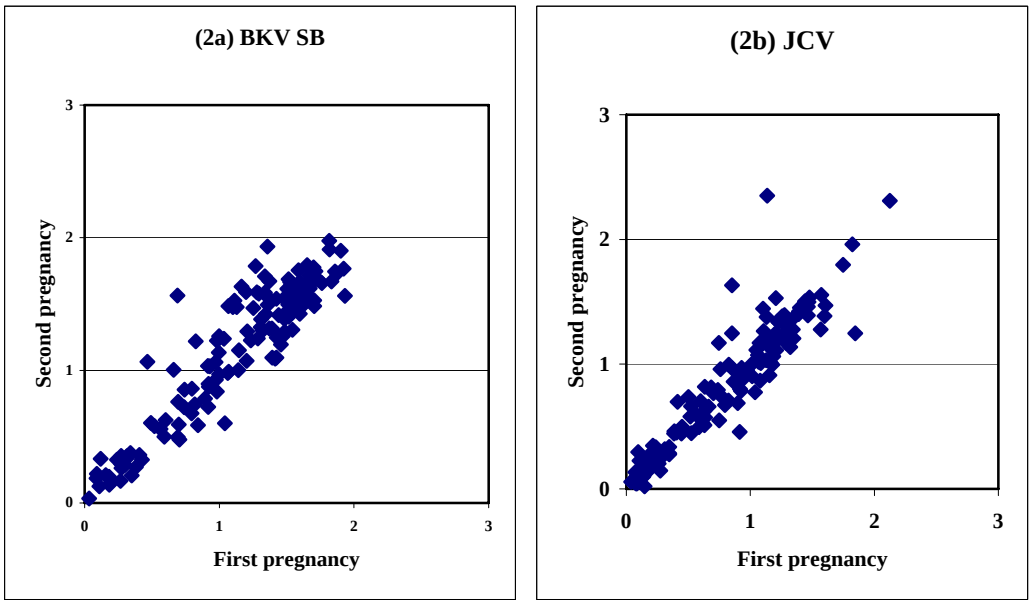

Fig. 2. BK and JC antibody levels are stable over time. Comparison of antibody levels between 2 pregnancies, up to 5 years apart, using scatter plots of antibody levels. Panel a, BKV SB antibodies(r=0.93). Panel b, JCV antibodies (r=0.94). Modified from reference[4]

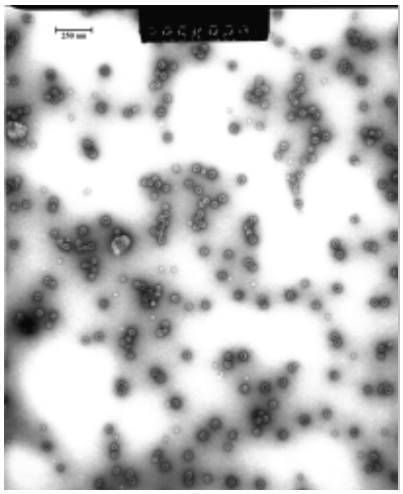

Figure 3. Negative-stain electron microscopy of BKV AS VLPs (x 28500) expressed in yeast (*S. cerevisiae*) cells.