Serological evidence of vertical transmission of JC and BK polyomaviruses in humans

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Vertical transmission of JC virus and BK virus has been investigated by few authors, with conflicting results. We performed a combined serological and genomic study of 19 unselected pregnant women and their newborns. Blood and urine samples were collected during each gestational trimester from the pregnant women. Umbilical cord blood, peripheral blood, urine and nasopharyngeal secretion samples were taken from newborns at delivery and after 1 week and 1 month of life. Polyomavirus DNA was detected by nested PCR. Polyomavirus IgG-, IgM- and IgA-specific antibodies were measured in maternal and newborn serum samples using a virus-like-particle-based ELISA method. BKV and JCV DNA were detected in urine from 4 (21%) and 5 (26%) women, respectively. BKV and JCV seroprevalences in the pregnant women were 84% and 42%, respectively. Using a rise in the IgG level or the transient appearance of an IgA or IgM response as evidence of infection in the newborn, we detected BKV and JCV infections in four (21%) and three (16%) newborns, respectively. Three infants had serological evidence of infection with both BKV and JCV. In two of the four possible BKV-infected newborns, the mothers seroconverted during pregnancy, while another mother was viruric and IgA seropositive. The mother of one of the three possible JCV-infected newborns was viruric and IgA seropositive; another mother was viruric. These results suggest JC virus and BK virus can be transmitted from mother to newborn during pregnancy or soon after birth.

INTRODUCTION

BK virus (BKV) and JC virus (JCV) are widespread human polyomaviruses (Walker & Padgett, 1983; Brown et al., 1975; Sundsfjord et al., 1990; Knowles et al., 2003), and their pathogenic role in the setting of immune deficiency has been clearly documented (Gardner et al., 1971; Padgett et al., 1971; Berger et al., 1998; Nickeleit et al., 1999; Boldorini et al., 2000; Hirsch & Snyderman, 2005). Primary infection usually occurs asymptotically (or with only mild respiratory symptoms) during childhood (Stolt et al., 2003), after which the polyomaviruses persist latently in various organs, mainly in the urogenital system (Boldorini et al. 2005), brain (Ferrante et al., 1995) and circulating leukocytes (Dörries et al., 1994). Reactivation of both viruses is common, and is frequently associated with asymptomatic viruria (Reese et al., 1975; Rziha et al., 1978; Jin et al., 1995). The natural history of infection is well established, but it is still not clear how BKV and JCV are transmitted, although the hypotheses include respiratory (Possati & Bartolotta, 1981; Goudsmit et al., 1982), oral–faecal (Bofill-Mas et al., 2000; Bialasiewicz et al., 2009; Vanchiere et al., 2005) and urinary transmission (Brown et al., 1975; Boldorini et al., 2005; Arthur & Shah, 1989). Furthermore, on the basis of the frequency of polyomavirus (PV) infection in childhood, and as has been previously demonstrated in the case of animal homologue polyomaviruses such as the murine (Zhang et al., 2005) and simian polyomaviruses (SV-40) (Patel et al., 2009), some authors have investigated the possibility of vertical transmission (Rziha et al., 1978; Taguchi et al., 1975; Borgatti et al., 1979; Coleman et al., 1980; Kunitake et al., 1995; Shah et al., 1980; Gibson et al., 1981; Pietropaolo et al., 1998), but with conflicting results.
A previous study (Boldorini et al., 2008) found no evidence of transplacental transmission in a population of 300 unselected pregnant women and their offspring. However, the findings were not conclusive as the evaluation was made only during the third trimester of pregnancy, using qualitative molecular methods to search for viral genomes in maternal blood and urine, and in umbilical cord blood. Furthermore, the presence of PV was not investigated serologically in either the mothers or the newborns.

In order to assess the possibility of vertical JCV and BKV transmission in more detail (including transplacental and/or perinatal transmission), we examined 19 pregnant women over the three trimesters of pregnancy, and their offspring during the first month of life. All the samples were analysed for JCV and BKV genomes by means of qualitative and quantitative molecular techniques, and serological data were obtained using virus-like particle (VLP)-based ELISA.

**RESULTS**

**Clinical data**

The 19 pregnant women had a median age of 29 years [interquartile range (IQR), 20–36] (Table 1). All but two pregnancies were physiological; woman no. 8 developed steatohepatitis while pregnant, and woman no. 17 was a renal transplant recipient who took immunosuppressive drugs throughout her pregnancy. The remaining women were healthy throughout their pregnancy. The gestational ages of the newborns at delivery ranged from the 37th to the 41st week (median, 40th week). Thirteen deliveries (68.4%) were eutocic. The six dystocic deliveries (31.6%) were resolved by means of a Caesarean section (five cases) or vacuum extraction (one case).

Nine babies were female and ten were male. The median weight at birth was 3370 g (IQR, 2780–4330 g). All the newborns except one were breastfed. All newborns were healthy at delivery but four infants had jaundice that rapidly and spontaneously cleared.

**Cytological analysis**

Cytological analysis failed to reveal decoy cells (DCs) in the 57 urine samples taken from the pregnant women.

**PCR analysis**

**Multiplex nested-PCR (n-PCR).** PV DNA was detected in 20 urine samples (20/57, 35.1%) taken from eight (42%) of the 19 pregnant women. Each woman contributed a median of two urine samples (range 1–3). JCV DNA was detected in five (26%) and BKV DNA in four (21%) of the 19 pregnant woman; one woman was secreting both JCV and BKV. In all cases the same PV was secreted throughout pregnancy. In seven of the eight viruric women, PV DNA was detected for the first time in the first trimester; one woman developed viruria in the second trimester. Six of eight women persistently shed virus in the urine. One

<table>
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woman cleared BKV after the first trimester, and one woman intermittently shed JCV.

PV DNA was not detected by multiplex n-PCR in any peripheral blood samples from the pregnant women, or in umbilical cord blood, peripheral blood, urine or nasopharyngeal samples taken from the newborns after 1 week and 1 month of life.

Serological analysis

Pregnant women. The overall seroprevalence of polyomaviruses in the pregnant women was 89% (17/19). Sixteen (84%) were BKV seropositive and eight (42%) were JCV seropositive; seven cases (37%) were seropositive for both viruses. Four (21%) pregnant women were BKV IgA seropositive and two (11%) were JCV IgA positive. With the exception of woman no. 1 who had a peak BKV IgA level of 0.753 optical density (OD) units (nearly ninefold higher than the cut-off point), IgA antibody levels were low and transient. Three of four BKV IgA-seropositive women were viruric and both JCV IgA-positive women were secreting JCV in the urine. Two (11%) pregnant women were BKV IgM seropositive. Woman no. 7 was seropositive at all trimester visits and secreted BKV in the urine during the first trimester. Woman no. 4 was IgM seropositive at the third trimester visit only. None of the pregnant women was JCV IgM seropositive.

Two pregnant women seroconverted for BKV, one woman in the second trimester (no. 8) and one woman (no. 4) in the third trimester. Woman no. 4 also mounted an IgM response.

Newborns. Fig. 1 shows the pattern of anti-JCV (a) and anti-BKV IgG (b) from delivery to 1 month of life for each newborn. Compared with the peak level at delivery and/or 1 week of age, the levels of JCV IgG declined at 1 month of age for all seropositive newborns except no. 4. For this infant, the IgG level rose from a nadir of 0.501 OD units at 1 week of age to 1.206 OD units at 1 month of age. The mother of newborn no. 4 was secreting JCV in the urine throughout pregnancy. Compared with peak levels, BKV IgG OD values fell by 1 month of age for all seropositive newborns with the exception of newborn no. 4. This infant had no detectable BKV IgG at birth and an OD value of 0.398 at 1 month of age. The mother of baby no. 4 seroconverted during pregnancy and was IgM seropositive at the third-trimester visit. We measured BKV and JCV IgA and IgM as additional serological markers of perinatal infection because these antibody classes cannot cross the placental barrier from mother to infant and their presence in a newborn is indicative of a de novo immune response. Newborn no. 8 was transiently BKV IgM positive at 1 week of age, newborn no. 2 was IgM positive at 1 month of age and newborn no. 3 was BKV IgM positive at delivery and 1 week of age. In all cases, the levels were low. No newborn had detectable BKV IgA. A low level of JCV IgM was transiently detected at 1 week of age in newborn no. 3 and at delivery in newborn no. 8. No newborn had detectable JCV IgA.

The virological and serological status of pregnant women and newborns in which an active BKV infection was proven or suspected are shown in Table 2; Table 3 gives equivalent data for JCV infection.

DISCUSSION

The vertical transmission of DNA and RNA viruses has been well documented in humans and animals. The modes of viral spread include passage across the placenta during pregnancy, birth-related processes and/or transmission soon after birth. The overall rates of vertical transmission can vary widely among viral species: the rates for varicella-zoster virus and human immunodeficiency virus range from 2 to 20% and from 13 to 30%, respectively, whereas that of cytomegalovirus infection has been reported as only 0.2% of newborns (Patel et al., 2009). The potential for vertical transmission of members of the family Polyomaviridae is controversial.

We attempted to clarify whether vertical transmission of human PV JCV and/or BKV occurs. To the best of our knowledge, this is the first molecular and serological study of BKV and JCV infection of women during pregnancy, and of their offspring during the first month of life.

The prevalences of BKV and JCV in urine of the pregnant women in our study were 21 and 26%, respectively. These results were not markedly different from those reported by Jin et al. (1993) and Markowitz et al. (1991). Conversely, Kalvatchev et al. (2008) reported a prevalence of 34.6% of BKV in the urine of pregnant women by amplifying the VP1 region using real-time PCR.

Our study provides serological evidence of vertical transmission of BKV and JCV in some newborns. Using a postnatal rise in the IgG level or the transient appearance of an IgM or IgA response in newborns as evidence of infection, we detected BKV and JCV infections in four (21%) and three (16%), respectively, of 19 newborns. Further support for vertical transmission in two of the four newborns with possible BKV perinatal infection was the observation that the mothers seroconverted during pregnancy, consistent with a primary infection. A third mother was viruric and IgA seropositive, a finding suggestive of viral reactivation. The mother of one of the three newborns with possible perinatal JCV infection was also viruric and IgA seropositive. Thus, evidence of active maternal infection was common among newborns with possible vertical transmission of BKV and JCV. However, BKV or JCV DNA was not detected in urine, peripheral or umbilical cord blood, or nasopharyngeal swab samples taken from these infants. Failure to document infection virologically in the newborns may be due to a low viral load, the timing of collection of the samples or the site of collection. Among the four newborns with possible
Fig. 1. Pattern of anti-JCV (a) and anti-BKV (b) IgG OD in newborns during the first month of life.
perinatal PV infections, three had serological evidence of infection with both BKV and JCV. Cross-reactivity in the VLP-based ELISA is unlikely, as we have previously shown that seroreactivity to JCV and BKV VLPs is entirely species specific (Viscidi et al., 2003b).

Primary PV infection is rarely reported in pregnancy (Borgatti et al., 1979; Coleman et al., 1980; Kunitake et al., 1995), and also in our study a definite primary infection by JCV and/or BKV could be hypothesized in only few cases. Pregnant woman no. 4 became positive for BKV IgG and IgM during the third trimester of pregnancy, and in her newborn IgG levels which were negative at delivery increased during the first week and first month of life. This serological pattern suggests that the woman may have had a primary infection during the later part of pregnancy and transmitted the infection to her newborn. Pregnant woman no. 8 seroconverted for BKV during pregnancy and her newborn was transiently BKV IgM seropositive. Although the BKV IgG level did not rise after birth, the presence of IgM is consistent with infection in the newborn. The possibility of primary infection leading to vertical transmission of a PV infection is supported by the experimental work of Zhang et al. (2005). They obtained transplacental transmission in 86% of the litters of pregnant BALB/c mice by intraperitoneal inoculation with high doses of murine polyoma virus during late pregnancy, mimicking an acute viral infection. However, whether these findings reflect what happens in pregnant women with acute primary infection needs further investigation.

In conclusion, our study indicates that maternal to fetal passage of both JCV and BKV polyomaviruses can occur in humans, although it is not a common means of spread for these viruses. Of note is that the study design cannot distinguish in utero transmission from transmission occurring at the time of birth or shortly thereafter. These findings need to be confirmed in a larger study, and a more intensive search for virological evidence of infection in newborns would further substantiate perinatal transmission.

**METHODS**

**Study population.** The study population consisted of 19 pregnant women and their newborns followed at the obstetrics and gynaecology unit of Azienda Ospedaliero-Universitaria Maggiore della Carità (Novara, Italy) between May 2008 and April 2009. Peripheral blood and urine samples were obtained from mothers during each gestational trimester. An umbilical cord blood sample was taken immediately after delivery. Peripheral blood and nasopharyngeal secretion samples were taken from newborns at 1 week and 1 month of life. A urine sample was obtained from 12 newborns at 1 week and from 14 newborns at 1 month. The study design was approved by the medical ethics committee of the Azienda Ospedaliero-Universitaria Maggiore della Carità and a written informed consent was obtained from the mothers.

**Sample processing.** Urine samples from mothers and infants were concentrated by centrifugation at 700 g for 10 min. The pellet was resuspended in 10 ml of PBS (pH 7.4). The sample from pregnant women was divided into two aliquots, which were either used immediately for cytological analysis as described below or stored at −20 °C for subsequent molecular analysis. The whole urine pellet from newborns was used for molecular analysis.

Blood samples were centrifuged at 700 g for 10 min, after which the serum was separated and stored at −20 °C. Nasopharyngeal secretions were collected from newborns by means of a Dacron fibre tip swab, and were immediately resuspended in 400 μl of PBS (pH 7.4).

**Cytological analysis.** Five millilitres of the resuspended urine pellets were cytocentrifuged onto two slides, which were fixed in 95 % ethanol, and air-dried. Both slides were then air-dried for about 30 min. Each slide was then placed in a slide-scale acid fixative, Cytological analysis of urine samples was performed by a single, blinded, experienced cytopathologist. Samples were evaluated under a phase-contrast microscope using a 40× objective.

**Sample processing.** Urine samples from mothers and infants were concentrated by centrifugation at 700 g for 10 min. The pellet was resuspended in 10 ml of PBS (pH 7.4). The sample from pregnant women was divided into two aliquots, which were either used immediately for cytological analysis as described below or stored at −20 °C for subsequent molecular analysis. The whole urine pellet from newborns was used for molecular analysis.

Blood samples were centrifuged at 700 g for 10 min, after which the serum was separated and stored at −20 °C. Nasopharyngeal secretions were collected from newborns by means of a Dacron fibre tip swab, and were immediately resuspended in 400 μl of PBS (pH 7.4).

**Cytological analysis.** Five millilitres of the resuspended urine pellets were cytocentrifuged onto two slides, which were fixed in 95 %

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**Table 2. Summary of virological and serological status of pregnant women and newborns with proven or suspected active BKV infection**

The remaining eight BKV IgG seropositive women were IgA and IgM seronegative and did not secrete BKV in urine during pregnancy. All the infants of these women had falling BKV IgG levels after birth. Falling refers to an OD value at 1 month that is lower than that at delivery and/or 1 week of age. +, Positive; −, negative.

<table>
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*Putative perinatal infection.

↑Rising refers to an OD value at 1 month of age which is higher than that at delivery and/or 1 week of age.
**Table 3.** Summary of virological and serological status of pregnant women and newborns with proven or suspected active JCV infection

The remaining two JCV IgG seropositive women were IgA and IgM seronegative and did not secrete JCV in urine during pregnancy. The infants of these women had falling JCV IgG levels after birth. Falling refers to an OD\textsubscript{405} value at 1 month which is lower than that at delivery and/or 1 week of age. +, Positive; −, negative.

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*Putative perinatal infection.
†Rising refers to an OD\textsubscript{405} value at 1 month of age which is higher than that at delivery and/or 1 week of age.

ethanol, stained with Papanicolaou stain and cytologically examined in order to identify the presence of DCs, which indicate active PV replication (Koos, 1979).

**DNA extraction and multiplex n-PCR for large T antigen (LT) amplification.** DNA was extracted from urine, serum and nasopharyngeal samples using commercial columns (Nucleospin Virus; Macherey-Nagel). PV DNA was detected by following a published protocol for a multiplex n-PCR targeting the LT region of the genome (Boldorini et al., 2008). The sensitivity of the PCR was estimated by amplifying serial dilutions of a BKV-positive sample. Each sample was tested by n-PCR three times and it was considered positive if the BKV region was amplified in at least two of three experiments (data not shown). DEPC-treated DNase/RNase-free water (Bioline) was used as the negative control. Positive controls were DNA extracted from PML brain tissue for JCV and from renal tissue of a subject with histologically proven BKV nephropathy for BKV.

**Serological analysis.** PV-specific antibodies were measured in the serum samples using VLP-based ELISA as previously described (Viscidi et al., 2003a). Briefly, 96-well microtitre plates (Maxisorb; Nunc) were coated with either purified BKV VLP protein (20 ng per well) or JCV VLP protein (20 ng per well) and then treated with a polyvinyl alcohol-containing blocking solution. The serum samples (diluted 1:100 for the detection of IgA and IgM, and 1:200 for the detection of IgG) were allowed to react with the antigen-coated plates, and antigen-bound immunoglobulin was detected using peroxidase-conjugated antibodies to human IgG, IgA or IgM (Southern BioTech). The specificity and the enzyme-labelled anti-IgA and anti-IgG conjugates were determined by an ELISA using wells coated with purified immunoglobulinomin, as described previously (Viscidi et al., 2003b). The anti-human IgM reagent was prepared by affinity chromatography and might react with IgM of other species but is immunoglobulin-class specific. Colour development was initiated by the addition of substrate (ABTS peroxidase substrate; KPL) and OD\textsubscript{405} was measured using an automated microtitre plate reader. Positive and negative control sera and sensitivity and reproducibility controls were included in each run.

The runs in which the replicate serum values fell outside the expected coefficient of variation were repeated. The results were recorded as OD values and as a categorical variable (seropositive or seronegative). The cut-off point for BKV and JCV IgG was an OD value >0.15 and was based on the distribution, excluding outliers, of OD values obtained with an independent dataset comprised of serum samples from young children. Cut-off points for IgA and IgM were set as an OD value greater than the mean +4 SD of the reactivity of the study samples, after excluding outliers. The IgA cut-off points for BKV and JCV were OD values >0.087 and >0.049, respectively, and for IgM the respective cut-off points were OD values >0.054 and >0.052, respectively.

**Statistical analysis.** The data were statistically analysed using the XLSTAT 09 program. The results are presented as median values and ranges.

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