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Presence of the newly discovered human polyomaviruses KI and WU in Australian patients with acute respiratory tract infection

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Abstract

Background: Currently, the role of the novel human polyomaviruses, KI (KIV) and WU (WUV) as agents of human disease remains uncertain.
Objectives: We sought to determine the prevalence of these viruses and their rate of co-detection with other viral respiratory pathogens, in an Australian population.
Study design: Polymerase chain reaction assays previously described were used to examine the presence of KIV and WUV in 2866 respiratory specimens collected from January to December 2003 from Australian patients with acute respiratory infections.
Results: KIV and WUV were present in our population with an annual prevalence of 2.6% and 4.5%, respectively. There was no apparent seasonal variation for KIV, but a predominance of infection was detected during late winter to early summer for WUV. The level of co-infection of KIV or WUV with other respiratory viruses was 74.7% and 79.7%, respectively. Both viruses were absent from urine and blood specimens collected from a variety of patient sources.
Conclusions: KIV and WUV circulate annually in the Australian population. Although there is a strong association with the respiratory tract, more comprehensive studies are required to prove these viruses are agents causing respiratory disease.

Keywords: Human polyomavirus; Acute respiratory infection; Respiratory viruses; Epidemiology; Molecular; Clinical; Emerging virus

1. Introduction

Intensive investigations over the last 6 years have led to the discovery of a number of new or emerging putative respiratory viruses, including human metapneumovirus (HMPV) (van den Hoogen et al., 2001), human coronaviruses, SARS (Ksiazek et al., 2003), NL63 (van der Hoek et al., 2004) and HKU1 (Woo et al., 2005), human bocavirus (HBoV) (Allander et al., 2005) and very recently, two new human polyomaviruses, KI virus (KIV) (Allander et al., 2007) and WU virus (WUV) (Gaynor et al., 2007). Their role as causative agents of respiratory disease has been conclusively demonstrated for some of these (HMPV, SARS, NL63), but for others their classification as a respiratory pathogen remains highly speculative in the absence of fulfilling Koch's postulates. This is particularly so for the newly discovered human KIV and WUV.

KIV and WUV were discovered independently in Sweden and the USA within months of each other. Allander et al. (2007) showed that KIV is phylogenetically related to other primate polyomaviruses in the early region of the genome, but had little homology in the late region. Similarly, by multiple sequence alignments of the predicted STAg, LTAg, VP1 and VP2 open reading frames, Gaynor et al. (2007) confirmed that WUV was clearly another novel polyomavirus that is most closely related to KIV. They also showed that WUV differed significantly from the other human polyomaviruses

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BK (BKV) and JC (JCV) in genome sequence and suggested that it is likely to have unique biological properties.

As a first step in elucidating the distribution and a possible role in human disease of KIV and WUV, we sought to determine the presence of these viruses in the population and their association with the respiratory tract. For this purpose we had at our disposal respiratory samples collected during 2003 from patients with acute respiratory tract infection (ARTI) that had been extensively tested for other known respiratory viruses. This sample cohort was investigated for the presence of KIV and WUV, seasonality, and rate of co-infection with each other or other common respiratory viruses.

2. Materials and methods

2.1. Respiratory specimens and nucleic acid extraction

We examined 2866 respiratory samples consisting of 2733 nasopharyngeal aspirates (NPAs), 91 bronchoalveolar lavage (BAL), 33 bronchial washings (BW) and 9 endotracheal aspirates (ETA) for the presence of KIV and WUV. Also, in consideration of the fact that BKV and JCV may be frequently found in urine and blood, we examined 215 urine samples, and 102 blood samples.

NPAs were collected from January 2003 until January 2004, from hospitalized patients or patients presenting for assessment at hospital emergency departments in Queensland, Australia, with ARTI. Patients ranged in age from 3 days to 95 years (mean = 9.2 years; median age = 1.38 years), and 76.5% of specimens were from children 5 years of age or younger. Nucleic acids were extracted from 0.2 mL of each NPA specimen using the High Pure Viral Nucleic Acid kit (Roche Diagnostics, Australia), according to the manufacturer's instructions.

To ensure that a diverse urine sample population was tested in this study, we included specimens that were obtained from a general hospital population and submitted for routine investigation of micro-organisms. Urine samples were collected between October 1999 and August 2005 from pediatric and adult patients ranging in age from 1 day to 76 years (mean = 16.9 years; median age = 24.6 years). Fifty-five samples were included from adult immunocompromised (bone marrow transplant) patients. Nucleic acids were extracted from 0.2 mL of each urine specimen with a QIAamp DNA blood mini kit (Qiagen, Clifton Hill, Australia). This procedure has been shown to be suitable for the extraction of viral DNA from urine specimens because of the high DNA yield and the removal of PCR inhibitors (Echavarria et al., 1998). Similarly, 102 blood samples collected between October 2004 and June 2005 for the monitoring of aspergillosis in 27 children with leukemia ranging in age from 2 to 16 years (mean = 7.6 years; median age = 6.0 years) were tested for KIV and WUV. A 200 μL volume of each blood sample was extracted as described for urine samples above.

2.2. Detection of KIV and WUV by PCR

Extracts were analyzed for the presence of KIV and WUV sequences by conventional PCR. A common PCR reaction mix was used for all conventional assays consisting of 1.25 units of QIAGEN HotStart Taq (Qiagen), 2.5 pmol of each primer, 0.625 μL of 10 mM dNTPs, 0.5 μL of 25 mM $MgCl_2$, 2.5 μL of 10× QIAGEN PCR buffer (Qiagen) and 2.5 μL of nucleotide extract made up to a 25 μL volume. Amplification was performed on an ABI GeneAmp 2700 instrument (Applied Biosystems Pty Ltd., Scoresby, Australia). For KIV, specimens were screened using primers POLVP1-39F and POLVP1-363R and positive results were confirmed with a second set of primers (POLVP1-118F and POLVP1-324R) as originally described by Allander et al. (2007). The KIV PCR assays utilized cycling conditions of 15 min incubation at 95 °C, followed by 40 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, followed by a final extension of 10 min at 72 °C. WUV was initially detected by conventional PCR using primers AG0044 and AG0045, as described by Gaynor et al. (2007), utilizing 15 min incubation at 95 °C, followed by 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, and a final extension of 10 min at 72 °C. PCR amplification products were realized by electrophoresis on 2% agarose gels and visualized with ethidium bromide staining. Positive results were confirmed using a WUV-specific real-time PCR assay, which was recently developed in our laboratory, incorporating a hydrolysis (TaqMan) probe (Bialasiewicz et al., 2007).

2.3. PCR analysis for other respiratory viruses

Samples had been previously tested for the presence of other significant respiratory viruses (respiratory syncytial virus (RSV), influenza virus A and B (INF A & B), parainfluenza viruses 1,2,3 (PIV 1,2,3), adenoviruses (ADV), HMPV) as previously described (Syrmis et al., 2004; Maertzdorf et al., 2004). All KIV and WUV positive specimens were further tested to determine the simultaneous presence of human rhinoviruses (HRV), human coronaviruses (HCoV) OC43, 229E, NL63 and HKU1 (Arden et al., 2006; Sloots et al., 2006) and HBoV. The presence of HBoV was determined by real-time PCR using primers STBoVP-1f (GGCAGAATTCAGCCATACTCAAA) and STBoVP-1r (TCTGGGTTAGTGCAAACCATGA) and a hydrolysis probe STBoVP-1probe (AGAGTAGGACCACAGTCATCAGACACTGCTCC-Yakima) targeting the HBoV VP-1 gene. All HBoV-positive results were confirmed by a second real-time PCR assay developed in our laboratory targeting the NP-1 gene (unpublished data).

2.4. Sequencing of KIV and WUV genomes

We sequenced the complete genomes of KIV detected in three patients in this study, and compared those sequences to KIV sequences available for the Swedish isolates (Allander