Molecular Epidemiology of KI and WU Polyomaviruses in Infants With Acute Respiratory Disease and in Adult Hematopoietic Stem Cell Transplant Recipients

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Polyomaviruses KI (KIPyV) and WU (WUPyV) were described recently in children with acute respiratory disease. The pathogenic potential of these human viruses has not been determined completely, but a correlation between immunosuppression and virus reactivation has been suggested. In the present study, the association between KI/WUPyV infection and immunosuppression was investigated using sequential nasopharyngeal aspirates from asymptomatic adult hematopoietic stem cell transplant recipients. In parallel, an investigation on the WU/KIPyV prevalence in children with acute respiratory disease was also carried out. Two of the 126 samples obtained from the 31 hematopoietic transplant recipients were positive for KIPyV (1 sample, 0.79%) and WUPyV (1 sample, 0.79%). Both samples were obtained 15 days after allogeneic transplantation and virus persistence was not observed in subsequent samples. In symptomatic children, 7 of the 486 nasopharyngeal aspirates were positive for WUPyV (1.4%) and 1 for KIPyV (0.2%). Single polyomavirus infection was detected in four patients, whereas the remaining patients were co-infected with respiratory syncytial virus (three patients) or adenovirus (one patient). The results suggest that WU/KIPyVs have a limited circulation in Italy and a low pathogenic potential in young children. Brief and asymptomatic infection can occur in hematopoietic transplant recipients. **J. Med. Virol.** 82:153–156, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: WU polyomavirus; KI polyomavirus; respiratory viruses; immunocompromised patients; acute respiratory disease

INTRODUCTION

The closely related KI and WU polyomaviruses (KIPyV and WUPyV, respectively) were first identified during 2007 in nasopharyngeal aspirates from patients with respiratory diseases [Allander et al., 2007; Gaynor et al., 2007]. In subsequent studies, these viruses were detected in a variable proportion of children with acute respiratory diseases and immunocompromised patients [Abed et al., 2007; Le et al., 2007; Lin et al., 2007; Foulogne et al., 2008; Neske et al., 2008].

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Despite detection of KIPyV and WUPyV in respiratory specimens, the link between the two viruses and acute respiratory diseases remains speculative; careful analysis is complicated by high co-infection rates with other well-characterized viral respiratory pathogens. Recently, a correlation between immunosuppression and reactivation of the two novel polyomaviruses has been suggested in immunocompromised patients [Mourez et al., 2009] and in AIDS patients at the molecular level [Sharp et al., 2009], but no evidence of a role of the viruses as opportunistic pathogens has been given. Overall, these data support the hypothesis that, in analogy with the BK and JC polyomaviruses, KIPyV and WUPyV can establish persistent infection, and that virus replication may increase in immunocompromised hosts. In this context, to clarify the pathogenic potential of the viruses, and the possible role of KIPyV and WUPyV as opportunistic pathogens, further studies on the extent of reactivation in patients with different immunodeficiency conditions may be useful.

In order to evaluate the association between KIPyV and WUPyV infection and immunosuppression, the prevalence and persistence of the polyomaviruses identified recently in sequential nasopharyngeal aspirates from asymptomatic adult hematopoietic stem cell transplantation recipients were examined retrospectively. In addition, a parallel investigation on the prevalence of WU/KIPyVs in pediatric patients with acute respiratory disease was carried out to obtain epidemiological data in Italy.

MATERIALS AND METHODS

A total of 612 archived respiratory samples (nasopharyngeal aspirates) were used in the present study. Samples were obtained during a recent survey of the prevalence of human metapneumovirus (hMPV) infection in symptomless adult hematopoietic stem cell transplant recipients (31 patients) [Debiaggi et al., 2006, 2007] and children (from 0 to 24 months old) with acute respiratory disease (486 patients) [Canducci et al., 2007]. In parallel, nasopharyngeal aspirates from 47 adult immunocompetent and asymptomatic patients were also tested as controls.

Hematopoietic stem cell transplant patients were recruited at the Division of Hematology, IRCCS Policlinico San Matteo (Pavia, Italy) regardless of respiratory symptoms, as described [Debiaggi et al., 2006, 2007]. Twenty-seven patients were allogeneic recipients, and the remaining four were autologous. A total of 126 sequential respiratory samples were obtained after informed consent from October 1st, 2004 to May 31st, 2006 and examined. From three to six samples from each patient were collected. Fifty-six samples were collected before transplantation (14 before admission to the hospital and 31 during the conditioning regimen), 60 and 21 were collected within 30 and 60 days after transplantation, respectively. At each time point clinical data were examined retrospectively.

Samples from pediatric patients were collected from October 1st, 2004 to September 30th, 2006 and from July 1st, 2007 to June 30th, 2008 at the microbiology laboratory of the Azienda Ospedaliera in Melegnano. All samples were from children from 0 to 24 months old with acute respiratory disease. The clinical samples were obtained as part of investigation of the standard hospital practice to assess the presence of human respiratory syncytial virus (hRSV), as described previously [Canducci et al., 2007].

Samples from adult immunocompetent patients were collected at the Respiratory Unit of Fondazione S. Maugeri, IRCCS, Pavia from January 31st, 2005 to January 31st, 2006. All samples were initially sent to the laboratory for routine respiratory infection investigations.

Viral nucleic acids were purified from clinical specimens using the Qiagen Viral extraction kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The genomic DNA extracts were identified for WUPyV using PCR and primers targeting the LTA gene, as described [Gaynor et al., 2007]. A second nested PCR reaction was also used on all samples to detect both KI and WU viruses. Primers, hybridizing on region of sequence conservation (VP2 gene) between the virus groups were: sense outer ATCTRTAGCTGGAGGAGCAGAG, sense, inner RTCAATTGCTGGWTCTGGAGCTGC, antisense, inner TCCACTTGSACTTCCTGTTGGG, and antisense, outer CCYTGGGGATTGTATCCTGMGG, yielding amplicon lengths of 336 and 276 bp for first and second round amplification reactions, respectively [Norja et al., 2007].

All of the specimens positive for KIPyV and WUPyV were also assayed for the presence of other respiratory viruses, including parainfluenza viruses (PIV 1–3), human rhinovirus (HRV), influenza A and B viruses, hMPV, hRSV, and adenoviruses, using a multiplex PCR strategy (Seeplex RV12 ACE Detection, Seegene, Rockville), and for bocaviruses and human coronaviruses, using protocols described previously [Canducci et al., 2007].

Finally, all KIPyV and WUPyV amplification products were sequenced bidirectionally, using BigDye Terminator v3.1 and an ABI 3100 sequencer (Applied Biosystems, Foster City, CA) to confirm amplification specificity and to allow genotyping. The present study was approved by the Istituto di ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo Ethical Committee, and informed consent was obtained from parents or guardians.

RESULTS

Two of the 126 samples obtained from the 31 hematopoietic transplant recipients were positive for KIPyV DNA (1 sample, 0.8%) and WUPyV DNA (1 sample, 0.8%) sequences (Table I). The polyomavirus DNA sequences were identified in samples collected in March 2005, 15 days after the allogeneic transplantation; these samples were obtained from one male with

TABLE I. Hematopoietic Stem Cell Transplantation Patients Positive for WU/KI Polyomaviruses

	Age (years)	Sex	Disease	Date of transplantation	Time of sample collection	Viral co-infections	Respiratory symptoms
KI	37	M	ALL	February 15, 2005	February 12, 2005	hMPV	None
					March 01, 2005 ^a	hMPV	None
					March 11, 2005	hMPV	None
					May 23, 2005	None	None
					March 08, 2005	hMPV	None
					March 15, 2005	hMPV	None
WU	38	F	AML	March 17, 2005	March 30, 2005 ^a	hMPV	None
					April 21, 2005	hMPV	Rhinorrhea
					May 23, 2005	None	Rhinorrhea

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia.

^aTime of WU/KI positive sample.

acute lymphoblastic leukemia, and one female with acute myeloid leukemia (37 and 38 years old, respectively). Other samples obtained sequentially from the same patients were negative for polyomavirus sequences, including those obtained 15 days before, and 30 and 60 days after allogeneic transplantation. Co-infection with hMPV was revealed in both specimens, but no respiratory symptoms were documented in these patients in parallel with KI/WUPyV detection in nasopharyngeal aspirates.

In pediatric patients, 7 of the 486 samples were positive for WUPyV (1.4%) and 1 for KIPyV (0.2%). The WU/KIPyV positive children ranged from 2 to 9 months old (mean age: 5.3 months). The positive cases were observed in winter (November–January 2004–2006) (Fig. 1A) and spring (March–May 2007–2008) (Fig. 1B). The polyomavirus DNA positive children had a diagnosis of bronchiolitis (three patients), bronchitis or broncopneumonia (four patients), and fever (one patient). Single infection with KIPyV and WUPyV was revealed in four of the eight positive cases, whereas the remaining four cases showed co-infection with hRSV (three patients) or adenovirus (one patient).

Finally, all of the 47 respiratory samples from immunocompetent and asymptomatic adult patients were negative for KIPyV and WUPyV DNA sequences.

DISCUSSION

This study addressed the molecular epidemiology of WUPyV and KIPyV in an Italian population of children with acute respiratory disease, and in adult hematopoietic stem cell transplant patients. In asymptomatic adult group, virus prevalence and persistence were evaluated by monitoring the infection at different time points, before and after allogeneic transplantation.

In hematopoietic transplant recipients, WUPyV and KIPyV sequences were found in 2 of the 126 nasopharyngeal samples, in both cases 15 days after stem cell transplantation. This suggests that virus infection and its detection in the respiratory tract may occur at the time of highest degree of immunosuppression. In our experience, this event was a spot, not followed by virus persistence, in contrast with the prolonged virus

shedding observed in symptomatic immunocompromised children reported by others [Le et al., 2007; van der Zalm et al., 2008]. Indeed, recent studies of immunocompromised adults or patients hospitalized for severe, life threatening, acute respiratory disease have suggested that infection with WUPyV and KIPyV occurs early in childhood, and that these viruses may establish persistent infections with possible reactivations paralleling respiratory disease or other clinical conditions [Le et al., 2007; Abedi Kiasart et al., 2008; Ren et al., 2008].

In the present study, samples were obtained from immunocompromised patients enrolled regardless of respiratory symptoms, as described previously

Fig. 1. Monthly distribution of respiratory samples from October 2004 to September 2006 (A) and from July 2007 to June 2008 (B).

[Debiaggi et al., 2006, 2007]. In the long-term survey of these patients [Debiaggi et al., 2007], the symptomless hMPV co-infection had a probable hospital origin and no late respiratory symptoms were observed.

The data obtained from pediatric patients with acute respiratory disease indicate that the WUPyV and KIPyV prevalence is low (1.4% and 0.2%, respectively) in Italy, comparable to that established in other countries [Le et al., 2007; Foullogne et al., 2008]. WUPyV and KIPyV infections can occur early in childhood, and these viruses are the only detectable pathogens in 50% of the infections. These data indicate that the novel human polyomaviruses play a role as respiratory pathogens or co-pathogens in infants.

Overall, the present study, using clinical samples obtained during a prolonged investigation on the prevalence of different viral agents in pediatric patients with acute respiratory diseases, suggests that WUPyV and KIPyV have a limited circulation and a low pathogenic potential as respiratory agents of infants. The occurrence of brief asymptomatic infection with the two polyomaviruses in hematopoietic stem cell transplant recipients indicates that large follow-up studies of different immunocompromised groups of patients are needed to elucidate whether polyomaviruses have any clinical relevance in immunodeficiency conditions.

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