Identification of Adenoviruses in Specimens from High-Risk Pediatric Stem Cell Transplant Recipients and Controls[▽]

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Received 9 August 2007/Returned for modification 26 August 2007/Accepted 25 October 2007

Adenovirus infection is an important cause of morbidity and mortality in stem cell transplant recipients. We report species and type-specific analysis from a prospective study of high-risk adenovirus infections following hematopoietic progenitor cell transplantation prior to, during, and after treatment with cidofovir, as well as species analysis of contemporaneously collected samples from control patients. Nine different adenovirus types representing all six recognized species were identified, and mixed infections were commonly found in this group of patients.

Adenovirus (ADV) infections are common in childhood. The 51 different currently recognized ADV serotypes are divided into six species (A through F) which have similar genetic and biological properties (3). ADVs commonly cause respiratory, gastrointestinal, and urinary tract disease. In the immunocompromised host, ADV infections can cause severe disease and death. In a recent review, Chakrabarti et al. (3) noted that ADV species B and C account for the majority of clinical isolates in hematopoietic progenitor cell transplantation (HPCT) recipients.

In this study, we determined the types of ADVs infecting seven pediatric stem cell transplant recipients prior to, during, and after treatment with cidofovir, as previously described (1), and compared them with 25 consecutive ADV isolates from nontransplant pediatric patients collected contemporaneously to determine the type-specific epidemiology of ADV infections in HPCT recipients and controls. A companion paper (1) examined the epidemiology of ADV infections in HPCT recipients and the effectiveness (if any) of cidofovir against these infections. This study was approved by the IRB of the Children's Memorial Research Center, Children's Memorial Hospital, Chicago, IL.

HPCT recipients and specimens. Patients underwent HPCT between 8 September 2003 and 1 December 2005 at our institution. Urine, stool, and throat samples were screened for ADV by viral culture (see below). Plasma was referred to ViraCor (Lee's Summit, MO) for quantitative ADV detection by PCR. Specimens were obtained weekly through the first 100 days (14 weeks) and then monthly until 1 year from the HPCT. A patient was defined as having a high-risk ADV infection (HRAI) if they had (i) a positive blood ADV PCR with or without symptoms, (ii) two or more positive ADV cultures from separate peripheral sites with or without symptoms, or

(iii) one positive peripheral culture for ADV plus compatible clinical evidence of ADV (1). As previously described, 7 of the 38 children developed HRAI and received cidofovir treatment (1). Two additional asymptomatic patients had ADV identified transiently from a single body site only without clinical symptoms and were not further studied.

Specimens from other patients. Twenty-five consecutive pediatric specimens from nontransplanted patients submitted to our virology laboratory during routine clinical care that grew ADV also had ADV species identification performed. These specimens included those from the respiratory tract (throat swabs and nasopharyngeal aspirates), urine, and stool specimens and were collected between July 2003 and April 2005.

ADV culture. A shell vial culture with R-Mix cells (Diagnostic Hybrids, Athens, OH) was performed on specimens from a respiratory source. Each specimen (0.2 ml) was inoculated onto the shell vial, centrifuged (2,000 rpm for 1 h), incubated at 37°C for 5 days, and stained with a Bartels viral respiratory screening and identification kit (Trinity Biotech Company, Carlsbad, CA) for influenza virus A and B, parainfluenza virus 1 to 3, ADV, and respiratory syncytial virus on day 2 and day 5.

Rhesus monkey kidney, A549, and MRC5 tube cultures were used for the recovery of ADVs from urine and stool specimens. Cultures that developed cytopathic effect were stained with the same Bartels reagents for identification. Cultures were held for 14 days before being discarded.

ADV species identification and typing. Total nucleic acids were extracted from 200 µl of culture isolates with a HighPure viral nucleic acid kit (Roche Diagnostics, Mannheim, Germany). Species identification of ADV was by PCR amplification and detection using an ADV consensus kit according to the manufacturer's instructions (Argene, Inc., Westbury, NY). Positive controls consisting of the plasmid containing the ADV sequences recognized by the primers were run in parallel according to the manufacturer's recommendations.

ADV type-specific identification was conducted at the Centers for Disease Control and Prevention by sequencing a partial region of the viral hexon gene, as previously described (12).

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[▽] Published ahead of print on 7 November 2007.

TABLE 1. Adenoviral infections in seven pediatric HPCT patients

Patient	No. of days posttransplant	Event ^a	Source of positive culture	Species/serotype(s) ^b	Patient	No. of days posttransplant	Event ^a	Source of positive culture	Species/serotype(s) ^b
1	6	+	Stool	D/29	5	115	+	Urine	E/4
	7	+	Urine	D/29		129	Cidofovir ended	N/A	
	12	+	Urine			0	+	Urine	
	12	+	Throat			0	+	Stool	A/31
	13	+	Stool			5	+	Throat	
	18	Cidofovir started	N/A			5	+	Urine	A/31
	25	+	Stool	D/29		5	+	Stool	
	36	Cidofovir ended	N/A			6	Cidofovir started	N/A	
	36	+	Stool			12	+	Throat	
2	81	+	Stool	D/29		12	+	Urine	
	75	+	Stool	C/2		12	+	Stool	
	82	+	Blood			19	+	Stool	
	82	+	Stool	C/2		21	+	Throat	
	83	Cidofovir started	N/A			26	+	Urine	
	88	+	Stool			26	+	Stool	
	89	+	Blood			26	Cidofovir ended	N/A	
	95	+	Stool	C/2		33	+	Throat	
	102	Cidofovir ended	N/A			33	+	Stool	A/31
3	134	+	Stool		6	0	+	Stool	F/41, A
	147	+	Stool	C/2		2	+	Urine	A/31
	32	+	Stool	C/2		13	+	Stool	
	60	+	Mouth			13	+	Urine	A/31
	83	+	Stool	C, B/3		21	Cidofovir started	N/A	
	83	+	Urine			40	Cidofovir ended	N/A	
	89	+	Stool		7	203	+	Urine	B/11, 34
	89	+	Urine	C/1		203	+	Stool	
	92	Cidofovir started	N/A			216	Cidofovir started	N/A	
	97	+	Stool			216	+	Stool	
	104	+	Stool			216	+	Urine	
	111	Cidofovir ended	N/A			216	+	Stool	
	132	+	Stool	B/3		222	+	Urine	
4	105	+	Throat	C/2		222	+	Urine	
	106	+	Stool	E/4		236	+	Urine	B/34
	110	Cidofovir started	N/A			236	Cidofovir ended	N/A	
	110	+	Urine	E/4		250	+	Urine	
	110	+	Throat			264	+	Urine	B/11, E
	110	+	Stool			282	+	Urine	
	110	+	Blood			291	+	Urine	B/11
	115	+	Throat			309	+	Urine	B/34

^a +, positive by PCR in blood specimens and by viral culture for specimens from other sources.

^b Species determined by using an adenovirus consensus kit PCR (Argene); selected isolates typed by PCR amplification and sequencing of partial hexon gene region (12).

Species identification and type-specific identification were conducted separately in a blinded fashion.

Results from samples from HPCT recipients. All seven patients who underwent HPCT had ADV isolated by culture from multiple specimens and thus met at least one of our criteria for HRAI. Our HRAI rate was 18% (1). Species identification and/or typing was performed on a convenience sample of 27 of the 60 viral isolates collected before, during, or after the initiation of cidofovir treatment (Table 1). Among these 27 positive cultures, all six species (A to F) and nine different ADV serotypes were identified. In all 27 cases where ADV species and type could be compared, the results were identical, although a second species was occasionally identified (Table 1).

Different species and types of ADV could be identified at various times during the treatment course. In three cases (pa-

tients 1, 2, and 5), the same species was isolated throughout the patient's course. In four cases (patients 3, 4, 6, and 7), more than one ADV species was identified.

ADV species from samples from other patients. ADVs from consecutively collected contemporaneous isolates from 25 different pediatric patients who were not transplant recipients were identified to species (Table 2). Only species A, B, and C were identified from these patients. Nineteen of these 25 specimens (76%) were from the respiratory tract. Species C accounted for >75% of these ADV isolates; the second most common species was B. Two species (B and C) were isolated from a single respiratory specimen. Thus, ADV species B and C comprised >95% of all ADV isolated from routine specimens obtained from nontransplanted patients.

ADV epidemiology and pathogenesis. Published studies documenting species or serotypes of ADVs from HPCT patients,

TABLE 2. Species and sources of the ADVs obtained from 25 clinical specimens from non-HPCT patients

ADV species	No. of specimens with species (%)	Source	No. of specimens from source
A	1 (4%)	Gastrointestinal	1
B	4 (16%)	Respiratory	4
C	19 (76%)	Respiratory	14
		Gastrointestinal	4
		Eye	1
B and C	1 (4%)	Respiratory	1

especially from North America, are limited. From Tennessee, Hale et al. (7) reported a 6% ADV infection rate among bone marrow transplant patients. Among the eight viruses (from five patients) typed, three were serotype 11, two were serotype 5, and one was serotype 7. A British group (2) reported a 21% infection rate in pediatric bone marrow transplant patients. Among the 68 ADVs studied, the most common serotypes were 1, 2, and 7. The ADVs from three lethal infections were serotypes 1, 5, and 32. There was no significant association between serotype and disease presentation. More recently, from Austria, Ebner et al. reported that 40 of 135 (30%) pediatric HPCT recipients were infected with ADV, predominantly serotypes 1 and 2 (76%) (5). Kroes et al. studied sequential isolates from the same patients. In their study of 83 patients, 40% had at least one positive ADV culture and 36% had more than one serotype identified. Interestingly, they reported serotypes 1, 2, and 31 as the dominant isolates, with type 31 occurring only in patients in whom multiple ADV isolates were obtained (9).

We reported that 7/38 (18%) of HPCT patients developed HRAI in the first year following transplant (1). From those seven patients, we isolated nine serotypes (1, 2, 3, 4, 11, 29, 31, 34, and 41) from 27 specimens typed. Thus, in our patient population, as has been previously reported (11), a broad range of ADVs were associated with infection, including type 41, which is not commonly seen in HPCT recipients (7).

Serotype 2 was isolated from three patients, and serotype 31 was isolated from two patients; all other isolates were isolated from only one patient. As seen in other studies (5, 9), serotype 2 was the most common isolate. Like Ebner et al. (5), we identified serotype 31 without another accompanying serotype (patient 5). Serotype 11 was isolated from urine (patient 7), as was seen in the studies by Hale et al. (7) and Kroes et al. (9). The importance of serotype 31 in early posttransplant infections had been suggested (9, 10). We found this serotype in two patients (5 and 6), both of whom had primary ADV infection (1) and were infected at the time of transplantation—in one case (patient 6) as part of a mixed infection. Patients 5 and 6 were the only two patients in our series who had HRAI at the time of transplantation (1).

The differences between the results of our series and those previously reported could be due to the numbers of samples studied, geographical differences, or study design. Most published studies have examined all ADVs identified, regardless of whether ADV was causing infection or contributing to disease (2, 5, 7, 9). A strength of our study was that we identified ADVs to species and type from patients with high risk of developing invasive disease.

Species epidemiology and pathogenesis. In a recent review, Chakrabarti et al. noted that ADV species B and C accounted for the majority of clinical isolates in HPCT recipients (3). In our HPCT patient population, species B and C were identified in four of seven patients: we identified species C in 6 of the 14 stool samples (out of a total of 30 ADV-positive stool samples) and only 1 of 12 urine samples (out of 21 ADV-positive urine samples) in which species were determined; conversely, we cultured species E in 3 urine samples but in only 1 stool sample. In our two patients with adenoviremia (patients 2 and 4), we cultured species C from the stool or throat and species E from urine and stool samples; however, species C was our most common stool isolate. Previous authors have reported disseminated infections in transplant recipients that were due to a wide variety of ADV species (2, 4, 5, 6, 11).

Patients 6 and 7 had different species of ADV detected at the onset of HRAI. Lion et al. (11) also found coinfection in 4/36 patients with ADV infection; 2 of the 4 coinfecting patients in their series had adenoviremia, and both died of disseminated ADV disease. None of our patients with HRAI died from ADV infection (1).

Four patients (patients 3, 4, 6, and 7) had mixed infections before cidofovir therapy. Thus, as was seen in the results of other studies, infections with multiple ADV types are not unusual (2, 5, 6, 7, 9, 11, 13, 14).

A recent study (6) reported the use of PCR grouping reagents to identify coinfections with two ADV species in children with flu-like symptoms. Our results also demonstrated the usefulness of these reagents in detecting mixed species in three cases (patients 3, 6, and 7) where simultaneous mixed serotype infection was not detected by DNA sequencing.

As shown by others (4, 8), species B and C were the most common ADVs (>95%) isolated from nontransplanted patients, and the range of sites of isolation (gastrointestinal, respiratory, and eye) was as expected (4). In contrast, ADV infections in the HPCT patients were complex; nine different ADV types representing all six recognized species were identified, and mixed infections were common. ADV infections in the HPCT patients were also systemic and persisted for months, even after the initiation of cidofovir treatment.

ADV disease in HPCT can result from primary infection or the reactivation of persistent or latent viruses (3). If primary, the ADV can be acquired either from the HPCT donor or from another source (exogenously). In patients with preexisting ADV antibodies (prior infection), subsequent ADV infection may be due to the reactivation of the patient's own strain or a new infection acquired from the HPCT donor or an exogenous source (3). Four of our patients with HRAI (1, 4, 5, and 6) had no detectable baseline antibodies against ADV, and thus probably had primary ADV infection (1, 3). In two of these four patients (patients 5 and 6), HRAI was detected before the receipt of HPCT, so the source of the infection was exogenous, but not the donor (1). Patients 1 and 4 acquired their primary infection either exogenously or from the HPCT donor. The remaining three patients (2, 3, and 7) either reactivated previous ADV infection or acquired a new serotype exogenously or from their donor (1). It has been suggested that since species C ADV infections are more likely to establish latent infections, they are also more likely to lead to disease via reactivation (3, 13). Indeed, patients 2 and 3 with species C infection had

detectable ADV antibodies at the time of transplantation and developed their infections 12 weeks after HPCT, suggesting reactivation (1).

It should be noted that our study methodology likely underestimated the full complexity of ADV infections in these patients. Virus cultures may have failed to grow more-fastidious ADV strains, and mixed infections may have been missed due to overgrowth of more rapidly growing strains. Moreover, our typing methodology examines only a limited region of the ADV hexon gene and would therefore fail to detect recombination events that occur outside of the target region. In addition, we did not perform genome restriction analysis, which could provide additional evidence of genetic variability among ADV isolates of the same type.

In summary, we found that a wide variety of ADV types can infect HPCT patients by reactivation or by newly acquired infections from donor or exogenous sources. This may further complicate efforts to prevent and control ADV infections in the transplant population.

We thank Tien Catdung and Yan Mu for technical assistance. We also thank Argene, Inc., for providing PCR reagents for the study.

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