Detection of Adeno-Associated Virus Viremia in Hematopoietic Cell Transplant Recipients

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Adeno-associated virus (AAV) is widely considered to be nonpathogenic, but the clinical epidemiology of this virus is limited. By use of polymerase chain reaction assays, we investigated the incidence and clinical significance of AAV viremia in a population of consecutive recipients of a hematopoietic cell transplant (HCT). Four (2.8%) of 145 patients developed AAV viremia after HCT. Viremia was low level and transient in all patients. Two patients were admitted to the hospital and died in proximity to AAV viremia (<7 weeks between diagnosis and death); however, AAV was not detected in tissue specimens obtained at autopsy. Thus, AAV does not appear to play a pathogenic role in organ-specific illness, even in a highly immunocompromised population.

The incidence and clinical impact of wild-type adeno-associated virus (AAV) infection among hematopoietic cell transplant (HCT) recipients is unknown. AAV, a parvovirus, establishes a latent infection by integrating into the host genome locus on chromosome 19 [1]. Replication competency can be reestablished by coinfection with a helper virus [2], typically an adenovirus or herpesvirus. AAV is widely considered to be nonpathogenic in immunocompetent individuals, and recombinant vectors based on AAV have generated considerable interest for gene therapies and contemporary vaccine approaches. However, knowledge of the incidence and clinical correlates of AAV viremia in immunocompromised patients has yet to be defined. We developed an AAV-specific real-time polymerase chain reaction (PCR) assay to investigate the spectrum of clinical disease associated with AAV viremia in a cohort of HCT recipients.

Materials and Methods

The study population was an unselected, prospectively accrued cohort of 145 pediatric and adult patients who received non–T-cell–depleted allogeneic or autologous HCT with myeloablative conditioning in 1998. Weekly plasma samples from these patients were collected from the day of stem cell infusion until day 100 after transplantation or until death, whichever occurred earlier. Samples were frozen at −80°C and evaluated in batches for evidence of AAV viremia. Patients gave informed consent, and the study was approved by the Fred Hutchinson Cancer Research Center institutional review board.

A quantitative PCR assay was developed to detect AAV subtypes 1–5, 7, and 8. We modified our *pan1/pan3* primer set according to previously published sequences for AAV1, 2, 3, 5, and 6 [3], and we designed specific Taqman probes for each set. Each primer/probe set was then tested on AAV1, 2, 3, 4, 5, 7, and 8. The set derived from AAV2 detected all AAV strains except AAV3, with similar or improved efficiency than the type-specific probes. AAV3 could be detected only with an AAV3-specific set. Therefore, the final PCR reaction contained primers and probes derived from AAV2 and AAV3.

DNA was extracted from 200 μL of plasma, using QIAmap96 DNA blood kits (Qiagen), and from tissue biopsy specimens, using EZ1 tissue kits on a BioRobot EZ1 workstation (Qiagen). DNA was eluted into 100 μL of AE buffer. A total of 10 μL of DNA was then used for each PCR reaction. Each 25-μL PCR reaction consisted of 12.5 μL of QuantiTect multiplex PCR master mix (Qiagen) and 833 nmol/L of Pan1 primer (AAC-TGG-ACC-AAT-GAG-AAC-TTT-CC); 416 nmol/L of the 2 modified Pan3 primers (Pan3a: AAA-AAG-TCT-TTG-ACT-TCC-TGC-TT; Pan3b: AAA-AAG-TCC-TTG-ACT-TCC-TGT-TT); and 100 nmol/L of each probe (probe 1: FAM-TTG-CAA-GAC-CGG-ATG-TTC-AAA-TTT-TAMRA; probe 2: FAM-CTG-CAG-GAC-CGG-ATG-TTT-GAA-TTT-TAMRA). EXO internal control was spiked into the PCR master mix to monitor for false-negative results, as described elsewhere [4]. The 7500 Fast Real-Time PCR system (Applied Biosystems) was used to test PCR reactions. The PCR cycling conditions were 95°C for 15 minutes to activate the polymerase, followed by 45 1-minute cycles at 94°C and 1 minute at 60°C.

The specificity of the PCR reaction was tested on 37 strains of adenoviruses, human genome, cytomegalovirus (CMV), Epstein Barr virus, varicella zoster virus, human herpesvirus (HHV) 6, HHV-8, herpes simplex virus 1, JC virus, BK virus, and parvovirus B-19. No cross amplification was detected. The assay detected all 7 strains of AAV (1, 2, 3, 4, 5, 7, and 8) obtained from the American Type Culture Collection and was sensitive to detect 10 copies of AAV consistently. The specificity was further confirmed by no detection of AAV in DNA extracted from 10 plasma samples, 26 peripheral blood mononuclear cell samples, and 23 saliva samples collected from healthy volunteers.

Bronchoalveolar lavage (BAL) and tissue specimens obtained at autopsy in the context of clinical care were processed in real time for pathogenic bacterial, viral, and fungal organisms, as described elsewhere [5], and residual samples were stored at −80°C. BAL and tissue specimens obtained at autopsy from patients who had AAV detected in their plasma were tested for AAV by quantitative PCR. BAL and lung tissue samples were additionally tested by PCR using published protocols for fungal pathogens [6, 7] and for 14 respiratory viruses [8, 9], including respiratory syncytial virus, adenoviruses, influenza viruses A and B, parainfluenza virus types 1–4, human metapneumovirus, rhinoviruses, and all 4 non–severe acute respiratory syndrome coronavirus subtypes.

Clinical, laboratory, and radiographic findings were correlated with AAV viremia events. All patients in our study were evaluated previously for CMV and HHV-6 plasma viremia [5], and a subset were evaluated previously for BK virus and adenovirus viremia [10, 11]. These data were explored for evidence of coinfection in our patients with AAV viremia.

Results

Of the 145 HCT recipients, 34% received an HLA-matched, related transplant; 6% received a mismatched, related transplant; 43% received an unrelated transplant; and 17% received an autologous transplant. Thirty-seven patients (26%) died within 100 days after transplantation. The mean age of patients undergoing HCT was 40.7 years; only 14 patients were younger than 21 years. Most (58%) were men. The underlying diseases were described elsewhere [5].

AAV was detected in plasma samples from 4 (2.8%) of 145 HCT recipients. The patient and virologic characteristics are outlined in Table 1, and the clinical correlates of AAV viremia are described in Table 2. Briefly, AAV was detected a mean of 47.5 days (range, 21–77 days) after transplantation. The average viral load was <1000 copies/mL, and the duration of viral detection was brief, ranging from 1 to 3 weeks (median duration, 1 week).

Two of the 4 patients with AAV viremia (patients 1 and 3) were admitted to the hospital with lung infiltrates, developed pulmonary failure, and died within 7 weeks and 3 weeks, respectively, from AAV detection. AAV was not detected in BAL or tissue

Table 1. Demographic and Virologic Characteristics for 145 Patients[a]

Characteristic	Value
Sex	
Male	84 (58)
Female	61 (42)
Mean age, years	40.7
Age, years	
<21	14 (10)
≥21	131 (90)
Myeloablative conditioning	145 (100)
Donor type	
Matched related	49 (34)
Mismatched related	9 (6)
Unrelated	62 (43)
Autologous	25 (17)
Deaths before day 100	37 (26)
Plasma samples tested, no.	1084
Samples per patient, median (range)	8 (1–18)
AAV-positive specimens, no.	6
AAV-positive patients, no. (%)	4 (2.8)
Duration between transplant and AAV detection, mean (range), days	47.5 (21–77)
Duration of viral detection, median (range), wk	1 (1–3)
Viral load, mean (range), copies/mL,	753 (50–2571)

Abbreviation: AAV, adeno-associated virus.

[a] Unless otherwise indicated, values represent no. (%) of patients.

specimens (from lung, gut, liver, kidney, spleen, and lymph nodes) taken at autopsy from either patient, and the postmortem examinations showed no cytologic changes suggestive of viral infection. The lungs in both patients showed evidence of diffuse alveolar damage.

Consistent with the finding of budding yeast forms in his BAL specimens, *Candida glabrata* was detected by PCR assays of BAL fluid from patient 1. No fungal or viral pathogens were detected in BAL or lung tissue specimens from patients 3. Of note, patient 1 had PCR evidence of BK virus and adenovirus viremia concomitant to AAV detection. Likewise, low-level HHV-6 viremia occurred in close proximity to AAV detection in patients 2, 3, and 4. CMV was not detected in any of these patients.

Other clinical findings were unsubstantial. Patient 4 reported nausea and diarrhea and was found to have hyperbilirubinemia and grade 3 graft versus host disease within one week of his AAV viremia event and died of unrelated causes on day 297. Patient 2 died on day 1517 of relapsed disease. Neither patient had abnormal pulmonary complications in association with AAV detection.

Discussion

AAV can be detected in the blood of HCT recipients, but the incidence is low, viremia is transient, and AAV does not appear to play a pathogenic role in organ-specific illness, even in a

Table 2. Clinical Characteristics of Adeno-Associated Virus (AAV) Viremia in Hematopoietic Cell Transplant Recipients

Case Patient	Age, y (Sex)	Underlying Malignancy (Transplant Type)	PTD of AAV-Positive Specimen (Viral Load, Copies/mL)	Duration of Viral Detection, d	Clinical Events in Week Before or After AAV Detection	Abnormal Radiographic or Laboratory Findings	GVHD, Grade (Location)	Copathogens Detected in Real Time	Copathogens Detected Retrospectively by PCR (Copies/mL)	Clinical Outcome and Autopsy Findings
1	48 (M)	CML (allo-MUR)	64 (1043); 71 (none detected); 79 (none detected); 87 (<50)	Up to 23	Admitted to the hospital with shortness of breath and hypoxemia	LLL infiltrate, pancytopenia, hypocellular bone marrow specimen	3 (skin, GI tract, liver)	BAL specimen showed budding yeast forms and gram-positive cocci; C. glabrata cultured from sputum	BAL specimen positive for C. glabrata; BK virus viremia (360 000) and adenovirus viremia (10 032) on d 64	Died on d 111 of pulmonary failure; DAD reported as cause of death at autopsy
2	48 (F)	MDSRA (allo-MUR)	21 (52.5)	<7	None	None	2 (skin, GI tract)	None	Low-level HHV-6 viremia (230) on d 21	Died on d 1517 of recurrent refractory anemia with excess blasts; no autopsy report available
3	40 (F)	AML (autologous)	77 (<50)	<7	Admitted to the hospital with dyspnea	Multiple patchy LUL and RLL infiltrates	None	BAL and lung biopsy specimens negative for infectious organisms; blood culture positive for Stenotrophomonas	Low-level HHV-6 viremia (23)[a] on d 63	Died on d 95 of pulmonary failure; autopsy findings included nonspecific destruction of small bronchioles, DAD, and leukemic relapse
4	18 (M)	ALL (allo-MR, second transplant)	28 (2571)	<7	Reported nausea and diarrhea as an outpatient; endoscopy positive for GVHD	Bilirubin peaked at 9.8 mg/dL with normal transaminase levels	3 (skin, GI tract, liver)	None	Low-level HHV-6 viremia (23) on d 28	Died on d 297 from P. jiroveci pneumonia and respiratory failure; family declined autopsy

Abbreviations: ALL, acute lymphocytic leukemia; allo, allogeneic; AML, acute myelogenous leukemia; BAL, bronchoalveolar lavage; C. glabrata, Candida glabrata; CML, chronic myelogenous leukemia; DAD, diffuse alveolar damage; GI, gastrointestinal tract; GVHD, graft versus host disease; HHV-6, human herpesvirus 6; LLL, left lower lobe; LUL, left upper lobe; MDSRA, myelodysplastic syndrome with refractory anemia; MR, matched related; MUR, matched unrelated; PCR, polymerase chain reaction; P. jiroveci, Pneumocystis jiroveci; PTD, posttransplantation day.

[a] Limit of detection of the assay is 23 copies/mL [5].

highly immunocompromised population. This study is important in helping to define the virologic characteristics and clinical epidemiology of AAV, a virus that is increasingly being used as a delivery mechanism for gene therapies and newer-generation vaccines.

AAV has been an attractive candidate for use as a viral vector, stemming from its ability to infect diverse human cell lines, the specificity of its integration into the host genome, and its presumed nonpathogenicity. However, little work has been done to evaluate the clinical epidemiology associated with wild-type AAV, and this virus has not been previously studied in highly immunocompromised patients. Although studies of wild-type AAV have historically focused on the serologic identification of prior or latent infection in hospitalized patients [12, 13], we used molecular diagnostic techniques to demonstrate instances of acute AAV viremia in a cohort of immunocompromised HCT recipients.

Although 2 patients died of pulmonary complications in proximity to their viremia (both <7 weeks), AAV was not detected in BAL or lung tissue specimens (or other tissue specimens) from either patient. Further, their autopsy specimens lacked any histopathologic evidence of viral infection. However, the number of cases is small, and it is unknown whether AAV itself would cause histologic changes in human tissue and whether histopathologic changes, if they occurred, would be those of the helper virus. Overall, the deaths are plausibly explained by *Aspergillus* pneumonia (case 1) and idiopathic pneumonia syndrome with leukemic relapse (case 3). Given the history of early *Aspergillus* infection in patient 3 and the potential for residual disease, this patient may also have died from fungal pneumonia, although we did not find PCR evidence of fungi in his lung tissue specimens.

It is interesting that, in addition to having AAV viremia, all 4 patients had evidence of concomitant viremia with HHV-6, BK virus, or adenovirus, although the viral load was low in 2 of the patients. The facilitated release in vitro of AAV from its host genome by proteins from helper herpesviruses or adenoviruses has been well described [2], yet there have been very few reports of in vivo codetection in symptomatic humans. In a small, 40-person epidemic of adenovirus conjunctivitis and pharyngitis in 1975, AAV was coisolated with adenovirus in secretions from 40% of patients [14]. Our study is the first to document codetection of these known helper viruses and AAV in immunocompromised patients. More than half of the patients in the cohort had viremia with other DNA viruses [5, 10].

The strengths of this study are the use of a consecutive unselected cohort with weekly prospectively collected plasma samples, quantitative assessment of AAV in BAL and tissue samples, and the broad-range of molecular tests to rule out other potential causes of disease. Limitations include the lack of other tissue specimens (eg, urine and stool) and the absence of some of the newly characterized human AAV serotypes from our PCR analyses [15]. Although our assay covered the most common serotypes used for AAV vectors, it is unknown whether the frequency or clinical manifestations of the uncovered subtypes would be any different from those of subtypes 1–8. In summary, low-level transient AAV viremia can be detected in HCT recipients, but the incidence is low, and AAV does not appear to cause any organ-specific illness, even in a highly immunocompromised population.

Notes

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