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Adenovirus Type F Subtype 41 Causing Disseminated Disease following Bone Marrow Transplantation for Immunodeficiency

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Adenovirus causes disseminated disease following bone marrow transplantation (BMT). We report a child who underwent T-cell-depleted BMT. Adenovirus subgenus F serotype 41 was detected antemortem by PCR in cerebrospinal fluid and postmortem in other tissues. Serotypes 40 and 41, associated with gastrointestinal disease, have not previously been implicated in disseminated disease.

CASE REPORT

A 5-month-old girl with Omenn's syndrome variant severe combined immunodeficiency (11) received rabbit anti-thymocyte globulin (rATG) at 5 mg/kg for 3 days and methylprednisolone, to eliminate damaging T-lymphocyte clones, and proceeded to a T-lymphocyte-depleted matched unrelated donor bone marrow transplant (BMT) following cytoreductive conditioning with busulfan (20 mg/kg); cyclophosphamide (200 mg/kg); and rATG (40 mg/kg), anti-LFA1 (2.6 mg/kg), and anti-CD2 (2.6 mg/kg) antibodies. Prior to BMT, adenovirus particles, subsequently identified as subgenus F, were identified by electron microscopy (EM) in pretransplant routine screening specimens of stool. Intravenous ribavirin (15 mg/kg/day) and oral immunoglobulin (150 mg/kg/day) were commenced. On day +26 post BMT, acute graft-versus-host disease (aGvHD) of the skin developed and was treated with methylprednisolone at 2 mg/kg/day for 3 days, and the dosage was reduced over 1 month. On day +64, an episode of gut aGvHD was treated with methylprednisolone at 1 mg/kg, tapered over 1 month, and cyclosporine. Chimerism studies of whole blood demonstrated donor alleles. By day +100, a small pericardial effusion was noted. By day +130, neurological abnormalities with sleepiness and poor head control developed. The aGvHD resolved, and cyclosporine was stopped. On day +143, 200 ml of pericardial exudate was drained; an adenovirus PCR assay performed on the fluid was negative, although adenovirus remained detectable in stool by EM. By day +155, she was irritable and drowsy, with head lag, brisk reflexes, ankle clonus, and apneic episodes. T-lymphocyte numbers increased to 163/mm^3, and stool had become negative for adenovirus by EM. Magnetic resonance imaging (MRI) showed cerebral atrophy with delayed myelination. Spinal cerebrospinal fluid (CSF) examination showed 10 leukocytes/mm^3 and 1.52 g of protein per liter, and adenovirus subgenus F was detected by PCR. High-dose ribavirin (30 mg/kg) was added, but the patient became febrile, opisthotonic, and unresponsive. Methylprednisolone (2 mg/kg with weaning over a week) was added. On day +180, a repeat cerebral MRI showed further atrophy consistent with postinfectious encephalitis. CSF examination demonstrated 1.3 g of protein per liter, but the CSF was negative for adenovirus by PCR. The progressive neurological deterioration with evidence of viral clearance suggested an exaggerated immunological response by engrafted T lymphocytes to disseminated virus. Despite further immunosuppression with methylprednisolone, rATG, anti-CD25, and anti-tumor necrosis factor alpha antibodies, the patient deteriorated and died on day +192. At postmortem, adenovirus subgenus F was found by PCR in the small and large bowels, the pericardium, the lungs, and the brain (Table 1) and identified as type 41 by DNA sequence analysis, although immunochemical analysis of these tissues for adenovirus was negative.

Histopathological examination of the brain showed widespread significant loss of neurons, accompanied by gliosis and areas of microcalcification, with marked loss of white matter in both cerebral hemispheres, although myelination appeared normal. The cerebellum showed diffuse moderate Purkinje cell loss of white matter. No features consistent with active adenovirus infection (specifically, no nuclear inclusions) were seen.

Antemortem CSF was tested by PCR for adenovirus subgenera A to F (10). Following detection of adenovirus DNA, a specific subgenus F PCR assay was used (1). Archived 20-μm-thick sections of fixed and paraffin wax-embedded tissue samples taken postmortem from six anatomical sites were dewaxed with xylene, washed with several changes of ethanol, and rehydrated. Nucleic acid was extracted and purified from five tissue sections of each type with the High Pure Viral Nucleic Acid kit (Roche Diagnostics, Lewes, United Kingdom). The primers used for PCR amplification of adenovirus hexon gene DNA were as described previously (2). A PCR assay was performed briefly comprising the following procedures: initial amplification by 25 cycles of PCR, nested PCR for 30 cycles with 1 μl of the initial reaction as the template on a LightCycler (Roche Diagnostics) with SYBR Green incorporated in the reaction mixture, and PCR product detection and identification by LightCycler software melting curve analysis. The ade-

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TABLE 1. Adenovirus loads determined by quantitative PCR in postmortem tissue samples

Tissue	Adenovirus load (no. of DNA copies/5 tissue sections)
Brain	5×10^3
Lung	4×10^4
Heart	Negative (<50)
Small bowel	6×10^3
Pericardium	2×10^2
Large bowel	2.25×10^5

novirus DNA copy number in the tissue sections was estimated by use of a standard curve generated from dilutions of an adenovirus PCR product, the concentration of which had been determined spectrophotometrically. Standard procedures to avoid PCR contamination were used.

The nested PCR products were sequenced bidirectionally with ABI Big Dye version 1.1 dye terminator chemistry and an ABI 310 Genetic Analyzer (ABI, Warrington, United Kingdom). Consensus nucleotide sequence data obtained were analyzed by comparison to all published viral sequences with the BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST/). For the five tissue samples found to contain adenovirus DNA, the sequence data allowed unambiguous identification of adenovirus subtype 41.

Adenoviruses are nonenveloped, double-stranded DNA viruses; six subgroups (A to F) have been established (9). Infection, spread by aerosolized droplets reaching the upper airways and conjunctiva, by the fecal-oral route, or nosocomially, is endemic in children. The incidence peaks between 6 months and 5 years of age. Most infections are self-limiting, mild gastrointestinal or respiratory illnesses; severe manifestations include hepatitis, nephritis, meningoencephalitis, and pneumonia, described in immunocompetent and immunocompromised individuals. After primary infection, the virus may remain latent in the peripheral blood, monocytes, upper airways, lungs, and gut (3, 6). Adenoviruses are increasingly important pathogens following allogeneic BMT, causing significant morbidity and mortality; children undergoing BMT are at particularly high risk (4), and the virus is often recoverable from multiple sites (3). Virus strains in serotype subgroups C and B more commonly cause disseminated disease after BMT (8). Adenoviruses in subgenus F are reported to cause only gastrointestinal disease and are rare in BMT patients, comprising only 8% of the adenovirus subtypes in one series in which none of the patients with adenovirus subgenus F died from adenovirus disease (8).

In our patient, adenovirus was detected by EM in stool samples before BMT and subsequently identified as belonging to subgenus F, serotype 41. Following BMT, aGvHD requiring immunosuppressive treatment with methylprednisolone and cyclosporine developed; both factors are associated with disseminated adenovirus disease (4). Initial investigations of a range of specimens, including blood, urine, and nasopharyngeal secretions following neurological deterioration failed to identify adenovirus by viral cultures, EM, immunofluorescence assay, or PCR assay, although adenovirus subtype 41 was subsequently detected in CSF by PCR. The clinical picture and MRI scan were consistent with a postinfectious immune response with emerging donor T lymphocytes causing tissue damage. Postmortem, adenovirus subgenus F type 41 was detected by PCR in the small and large bowels, as expected, but also in pericardial, lung, and brain tissue samples. Adenovirus type F is difficult to grow in routine cell cultures, and EM is less sensitive than PCR, possibly explaining the initial failure to detect adenovirus in nonenteric specimens. T-lymphocyte-mediated immunity is important for recovery after acute infections. The patient had donor T-lymphocyte engraftment, and it is likely that T lymphocytes cleared the adenovirus from the stool and brain but caused postinfectious encephalitis, a phenomenon previously described in BMT patients (5). Adenovirus was not seen on histological examination of the brain because it is likely to have been cleared by functioning T cells, which caused a postinfectious encephalitis. The more sensitive detection method of PCR was able to detect a small number of viral particles in a postmortem specimen. The PCR assay detects adenovirus strains of other subtypes, and adenovirus type 41 was confirmed by DNA sequencing. Contamination by adenovirus type 41 DNA is unlikely as tissue specimens were handled with fresh microtome knives for each specimen and the PCR was performed in a laboratory that does not routinely process stool specimens. Standard procedures to avoid PCR contamination were also used. It is possible that the knife used in the original postmortem was contaminated with adenovirus subgenus F type 41 from the gut and subsequently transferred the virus to lung, pericardium, and brain tissue samples. However, the high number of viral genome equivalents per DNA extraction from other tissues makes this unlikely (Table 1). Although the pericardium was positive for adenovirus subgenus F type 41 by PCR, the heart tissue was negative, suggesting that cross-contamination is unlikely. Finally, antemortem CSF was adenovirus subgenus F type 41 positive by PCR 33 days prior to death.

The finding of adenovirus DNA in nonenteric tissues from a patient with an adenovirus type 41 infection raises the possibility of coinfection with other adenovirus serotypes (5, 8). In our patient, adenovirus subtype 41 was detected in stool prior to BMT. It is unlikely that a different adenovirus was acquired from the donor as T-lymphocyte-depleted marrow was given. However, T-lymphocyte depletion was done by the use of in vitro Campath-1 M and complement, and it is possible that viral DNA, present in the harvested marrow, was therefore present in the T-lymphocyte-depleted marrow product. Nevertheless, PCR assay of postmortem tissue unequivocally demonstrated adenovirus type 41 in pericardium, lung, and brain samples, as well as in the gastrointestinal tract, which is likely to have disseminated from the gastrointestinal tract rather than to have been acquired from the donor marrow.

The recent introduction of routine surveillance for adenovirus by PCR assay enables early detection of asymptomatic infection and early and aggressive treatment with new antiviral agents such as cidofovir (7), methods not routinely available when our patient was treated. Dissemination of subgenus F type 41 has not previously been reported to our knowledge. Clinicians should be aware that the presence of adenovirus

type 41 in the stool of high-risk patients may lead to the dissemination of this virus.

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