Disseminated Adenovirus Disease by Multiple Adenovirus Serotypes following Allogeneic Hematopoietic Stem Cell Transplantation

Adenovirus (AdV) is recognized increasingly as an emerging pathogen causing relevant morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-SCT), especially in pediatric and T cell-depleted allo-SCT recipients [1]. Recent progress has been focused on the importance of monitoring of plasma AdV DNA loads, which enables early diagnosis, prediction of disseminated disease, and evaluation of antiviral therapies [2,3]. However, the epidemiologic and virologic details about AdV serotypes and infection mode are still not well understood. Recently, systematic analysis of AdV serotypes after SCT has revealed sequential occurrence of multiple AdV serotype infections in pediatric allo-SCT recipients [4]. Here, we report an adult case of severe disseminated AdV disease by concurrent infection of 2 AdV serotypes, AdV 3 and AdV 34, after allo-SCT for myelodysplastic syndrome (MDS) with autoimmune hepatitis (AIH). The clinical relevance of multiple AdV infections is discussed.

A 35-year-old female was diagnosed as having MDS of refractory anemia with excess of blast-2 (RAEB-2). Interestingly, the blasts were characterized as B lineage lymphoblasts by the cell surface marker expression pattern and the IgH clonal rearrangement. Six months after the diagnosis, she developed AIH with progressive neutropenia and an increment of lymphoblasts. After 0.75 mg/kg/day of prednisolone (PSL) administration, both AIH and hematologic abnormalities were remarkably improved, suggesting that AIH should be a paraneoplastic autoimmune phenomenon. To the best of our knowledge, AIH is firstly described as an MDS-associated autoimmune disorder. Lymphoid involvement is also a rare event in MDS [5], and might cause a peculiar immune derangement attributable to the development of AIH.

Four months later, she received a bone marrow transplantation (BMT) from an HLA 5/6-matched unrelated donor following conditioning with cyclophosphamide (CY) and 12 Gy of fractionated total body irradiation (TBI). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine (CsA) and a short course of methotrexate (MTX). After engraftment on the day 24, gross hematuria appeared and followed by bilateral hydronephrosis and renal failure (Figure 1). The rapid immunochromatography test detected AdV in her urine. On the day 34, she was admitted to the intensive care unit, because of severe respiratory, cardiac, and renal failure. CsA was replaced with 0.5 mg/kg/day of PSL because of renal failure. AdV was detected in blood and bronchoalveolar lavage fluid (BALF) by polymerase chain reaction (PCR) analyses, and blood plasma AdV DNA load was 5×10^6 copies/mL, which was detected by real-time quantitative PCR as previously described [2,6]. Ganciclovir was continuously used, because cidofovir is unapproved for use in Japan. Anti-AdV high-titer intravenous immunoglobulin was also administered, and PSL was rapidly reduced with no signs of acute GVHD. Around day 45, however, hemorrhagic colitis and severe hepatitis appeared. Continuation of intensive supportive care

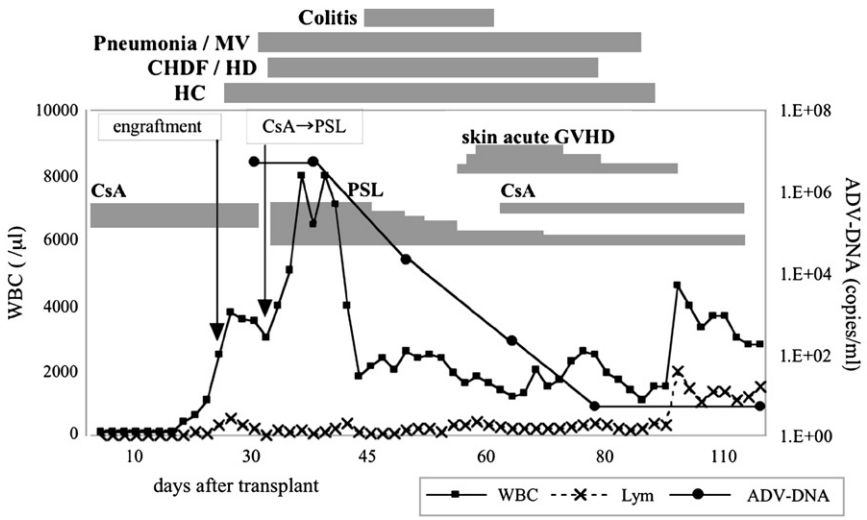

Figure 1. Clinical and virologic course after bone marrow transplantation. Abbreviations: WBC, white blood cells; Lym, lymphocytes; CsA, cyclosporine; PSL, prednisolone; HC, hemorrhagic cystitis; CHDF, continuous hemodiafiltration; HD, hemodialysis; MV, mechanical ventilation.

Table 1. *Sequences of Serotype-Specific Primer Sets for PCR Detection of Some AdV Serotypes*

Serotype (Subspecies)	GenBank ID	Region Gene/Product	Oligonucleotide Squence 5′-Forward-3′/ 5′-Reverse-3′	PCR Product Size (bp)
AdV (qRT-PCR)	NC001405	Hexon protein	AD185S / AD185A: 5′-tccagcaacttcatgtccatgg-3′/ 5′-tcgatgacgccgcggt-3′ TaqMan probe: 5′-(FAM)-cccatggacgagcccaccct-(TAMRA)-3′	
AdV 1 (C)	AF534906	L4/hexon protein	Outer (1st): 5′-ttctgatacccaaactgaaggcaatcc-3′/5′-gttttgcttggtttgtatgaaatgtgagtatcag-3′	369
			Inner (2nd): 5′-tttttgccgatcccacttatcaacc-3′/ 5′-tgctactagtataccttgaccccca-3′	176
AdV 2 (C)	J01917	L4/hexon protein	Outer (1st): 5′-gctacaaataggatcagacaatgcagaa-3′/ 5′-cacttttggtttagtagcattgccttg-3′	319
			Inner (2nd): 5′-cagtggaacgaagctgatgctaatgcgg-3′/ 5′-agaggcacccctttttcatccgg-3′	149
AdV 3 (B1)	AY599834	L3/hexon protein	Outer (1st): 5′-tacaacaaatggggacaatgcagtaact-3′/ 5′-agtttcaacccctccttcggt-3′	352
			Inner (2nd): 5′-caaattgggaaagacattaccactactgaag-3′/ 5′-ccccctttatgtttgtaggtcttgc-3′	209
AdV 5 (C)	AY601635	L3/hexon protein	Outer (1st): 5′-ttaaggaaggtaactcacgagaactaatgg-3′/ 5′-gtctctgtattaatcacacctcccagt-3′	358
			Inner (2nd): 5′-ggaaggtaactcacgagaactaatgggcc-3′/ 5′-ccgccagaacacccatattacccgt-3′	128
AdV 7 (B1)	AY594255	L3/hexon protein	Outer (1st): 5′-atagttacagcaggagaagaaagagcagt-3′/ 5′-acatctccttcggttggttttacttttct-3′	338
			Inner (2nd): 5′-aaagacattactgcagacaacaagccc-3′/5′-gctggtttaagagctctacctccaaa-3′	134
AdV 11 (B2)	AF532578	L3/hexon protein	Outer (1st): 5′-aatacaactggtgaggaacacgtaacag-3′/ 5′-ccgcatcaaaaaactccatgtcgatatcat-3′	388
			Inner (2nd): 5′-tggaagtttcagatgaagaaagtaaaccga-3′/5′-caaaggacccgtagcatggtttc-3′	168
AdV 34 (B2)	AY737797	L3/hexon protein	5′-gaaggtcctaaaccaatctatg-3′/ 5′-gatgttgttagtgccatcg-3′	225
AdV 35 (B2)	AY271307	L3/hexon protein	5′-atgaaacagaggagaaaactg-3′/ 5′-agatgggtttagattcgtttt-3′	129

gradually improved her conditions with a slow restoration from lymphopenia, and a reduction of AdV DNA load. AdV DNA in plasma became undetectable on day 67, and hemodialysis and mechanical ventilation were finally withdrawn. She maintained wellness with complete remission 1 year after BMT.

Among Japanese adult recipients, AdV 11 (Species B) is most commonly incriminated as hemorrhagic cystitis [6], which rarely causes severe AdV disease. We thus performed PCR-based AdV serotyping. Serotype-specific primer sets were designed on the hexon regions of 8 AdV serotypes (AdV 1, 2, 3, 5, 7, 11, 34, and 35). Nested PCR analyses were executed using outer and inner primers for detection of AdV 1, 2, 3, 5, 7, and 11. The sequences of the primers are summarized in Table 1. As a consequence, we identified 2 serotypes, AdV 3 (subspecies B1) and 34 (subspecies B2), in both blood and BALF around days 35-50, whereas AdV 1, 2, 5, 7, 11 and 35 were not detected.

Adenoviruses are subdivided into 6 subspecies and 51 serotypes. Especially, species B and C have been the major causes of AdV infections in allo-SCT recipients, but some regional or racial differences exist in epidemiologic serotype distribution [6-8]. AdV serotyping is not necessarily required for the diagnosis of AdV infection, and has been performed only in limited cases. Recently, a sustained analysis of AdV serotypes demonstrated sequential emergence of multiple AdV serotypes among pediatric recipients who underwent allo-SCT [4]. More than 1 serotype could be detected sequentially in 36% of AdV infections after allo-SCT. Additionally, single serotype infections had a lower survival rate than multiple serotype infections, most of which were continually observed in

nonmalignant disorders accompanied with immune dysfunction. This study depicted the complex nature of AdV infection after allo-SCT.

On the other hand, the clinical relevance of multiple infections has not been investigated so far in adults. Coinfection of AdV species B and C was also observed in adult recipients [8], and none of the cases with B and C species viremia showed serious infectious complications. We added a new case of mixed infection, in which 2 AdV serotypes, AdV3 and AdV34, were identified by serotype-specific PCR analyses. Our case has been narrowly rescued, but the severity was quite extensive and differs from the favorable outcome of either sequential AdV infections in pediatric recipients, or mixed AdV viremia in adult recipients. A prolonged immunocompromised state due to MDS with the lymphoid abnormality and pre-BMT immunosuppressive therapy to AIH may attribute to both multiple AdV infections and the severe clinical course. The clinical importance of multiple AdV infections may depend on several factors such as patient populations, underlying diseases, stem cell sources, GVHD statuses, and AdV serotypes.

Ribavirin is 1 of the treatment options of AdV disease, but the clinical response varied considerably in contrast to cidofovir. Considering that antiviral activity of ribavirin is relatively specific for AdV species C and highly serotype-dependent [9], this type of infection might contribute to the variable outcome of ribavirin therapy. Moreover, this phenomenon would be important for verifying the role of reactivation of latent or persistent viruses in AdV infection after allo-SCT and development of more efficient immunotherapeutic interventions. Serotype-specific PCR analysis performed in this case may be useful for validation of this mode of infection as well as other genotypic analyses [10,11]. Further investigation of simultaneous and/or sequential AdV infections could provide new insights about the pathogenesis and management of AdV infections after allo-SCT.

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