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CASE REPORT

Temporal and Spatial Compartmentalization of Drug-Resistant Cytomegalovirus (CMV) in a Child with CMV Meningoencephalitis: Implications for Sampling in Molecular Diagnosis

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We describe a case of antiviral-resistant cytomegalovirus meningoencephalitis occurring after hematopoietic stem cell transplantation. Antiviral-resistant cytomegalovirus was identified in blood 16 months earlier. However, wild-type cytomegalovirus was evidenced in blood when the meningoencephalitis was diagnosed. Treatment of meningoencephalitis should be adapted to all previously identified resistance mutations in any compartment.

CASE REPORT

In July 2008, a 21-month-old patient was diagnosed with an inherited immune deficiency, a major histocompatibility complex (MHC) class II expression deficiency caused by homozygous mutation in the *RFXANK* gene. He had a history of successive herpes simplex virus 1 (HSV-1) gingivostomatitis successfully treated with acyclovir (ACV). Positive human cytomegalovirus (CMV) serology suggested a previous undiagnosed episode(s) of CMV viremia, which spontaneously resolved. At diagnosis (July 2008), an intravenous replacement of polyvalent immunoglobulins and an oral ACV-based prophylaxis against HSV-1 infections were implemented.

In June 2009, an episode of asymptomatic CMV viremia was treated with intravenous ganciclovir (GCV). In July 2009, the patient received allogeneic hematopoietic stem cell transplantation (HSCT) using a HLA-mismatched unrelated cord blood unit and a conditioning regimen associating fludarabine, melphalan, and alemtuzumab. At day 3 post-HSCT, a CMV disease was suspected because of concomitant diarrhea, fever, and high CMV viremia. Because intravenous foscarnet (FOS) (90 mg/kg of body weight administered twice daily) failed to control the CMV viremia, GCV was added (5 mg/kg administered twice daily). FOS was replaced with cidofovir (CDV) (in addition to GCV) between September and December 2009 because of the diagnosis of a concomitant adenovirus infection. In November 2009, a CMV genotypic resistance test was performed by *UL97* and *UL54* viral gene direct sequencing (as previously described [1]), but no resistance mutation was evidenced (Table 1). The monitoring of GCV plasma exposure revealed extremely low trough GCV levels (<0.1 mg/liter); adequate pharmacokinetic exposure (trough GCV level ≥ 0.5 mg/liter) was achieved by increasing the daily GCV doses to 3 mg/kg/6 h. Finally, because of the persistence of uncontrolled CMV viremia despite GCV-plus-FOS bitherapy, artesunate was added in December 2009. CMV viremia resolved in January 2010, and the antiviral multitherapy was replaced with a valganciclovir (VGCV)-based maintenance treatment. At the same time, HSCT graft rejection was diagnosed in our patient (MHC class II expression < 3% in T lymphocytes).

In March 2010, the patient experienced a new episode of CMV viremia. A CMV genotypic resistance test showed the following mutations: M460I in UL97 phosphotransferase, conferring resistance to GCV, and L545S in UL54 DNA polymerase, conferring resistance to GCV and CDV. However, CMV viremia could be promptly controlled with a GCV-plus-FOS treatment.

In January 2011, a new episode of CMV viremia occurred under conditions of VGCV-based maintenance treatment. The genotypic resistance test showed the repopulation of the blood with wild-type strains (Table 1). After 2 weeks of FOS therapy, which successfully controlled the viremia, a treatment with valaciclovir (VACV)—instead of VGCV—was initiated because of the risks of hematological toxicity in patients with prolonged VGCV exposure.

In September 2011, rapidly progressive vision loss was diagnosed. The ophthalmological examination found bilateral pale papilla, without signs of viral retinitis. Cerebral magnetic resonance imaging showed abnormalities consistent with encephalitis. CMV PCR was negative in blood but positive (3.8 $\log_{10}$ copies/ml) in the cerebrospinal fluid (CSF). A CMV genotypic resistance test performed in a CSF sample evidenced H520Q and L545S mutations in UL97 phosphotransferase and UL54 DNA polymerase, respectively, conferring resistance to GCV and CDV. A GCV-plus-FOS bitherapy failed either to improve the clinical symptoms or to decrease the CMV load in CSF (3.9 $\log_{10}$ copies/ml), despite adequate GCV pharmacokinetic exposure. Because of the lack of efficacy of the antiviral treatment and its poor hematological and renal tolerance (deep neutropenia requiring daily filgrastim administration and severe tubulopathy), the GCV-plus-FOS bitherapy was stopped in October 2011. Because (i) no other virolog-

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TABLE 1 Evolution of the CMV PCR and genotypic resistance tests in blood and cerebrospinal fluid samples[a]

	Blood result				Cerebrospinal fluid result				Antiviral treatment	
		Amino acid change associated with CMV resistance to antiviral drugs[f]				Amino acid change associated with CMV resistance to antiviral drugs				
Date(s)	CMV PCR[b]	Date	UL97 PT[c]	UL54 DP[d]	CMV PCR[b]	Date	UL97 PT[c]	UL54 DP[d]	Drug(s)	Duration
July 2008–June 2009	−				−				Oral ACV	11 mos
June 2009	+ (3.9 → <2.7)				−				GCV	2 wks
June–July 2009	−								Oral ACV	4 wks
July–August 2009	+ (3.2 → 5.4)								FOS	3 wks
August–September 2009	+ (5.4 → 3.9)								FOS + GCV	3 wks
September–December 2009	+ (3.9 → 3.6)	November 2009	0	0					CDV + GCV	14 wks
December 2009	+ (3.6 → 5.8)								FOS + GCV	2 wks
December 2009–January 2010	+ (5.8 → <2.7)								FOS + GCV + artesunate	4 wks
January–March 2010	−								VGCV	2 mos
March–April 2010	+ (2.9 → 4.4)								VGCV	2 mos
May 2010	+ (4.4 → <2.7)	May 2010	M460I	L545S					FOS + GCV	2 wks
June 2010–January 2011	−								VGCV	7 mos
January 2011	+ (3.3 → <2.7)	January 2011	0	0					FOS	2 wks
January–September 2011	−								VACV	9 mos
September–October 2011	−				+ (3.8 → 3.9)	September 2011	H520Q	L545S	FOS + GCV	5 wks
October–November 2011	−				+ (3.9 → 4.9)				VACV	9 wks
November 2011–June 2012	+ (3.4 → 3.7)	February 2012[e]	0	0	+ (4.9 → 4.3)	February 2012	H520Q	L545S	VACV	7 mos
		February 2012[e]	H520Q	L545S						
		May 2012	M460I	L545S						
June–August 2012 (death)	+ (3.7 → 5.3)	June 2012	M460I	L545S	+ (4.3 → 3.2)				FOS + GCV	5 wks

[a] CMV, cytomegalovirus; UL97 PT, UL97 phosphotransferase; UL54 DP, UL54 DNA polymerase; ACV, acyclovir; GCV, intravenous ganciclovir; FOS, foscarnet; CDV, cidofovir; VGCV, valganciclovir; VACV, valaciclovir; i.v., intravenous.

[b] In cases of positive CMV PCR results, results of the PCR at the initiation and at the end of each antiviral regimen are indicated in parentheses (expressed in $\log_{10}$ copy numbers/ml).

[c] In all genotypic resistance tests, the following amino acid changes relative to natural polymorphisms of CMV UL97 phosphotransferase were isolated: Q19E, N68D, S108N, and I244V.

[d] In all genotypic resistance tests, the following amino acid changes relative to the natural polymorphism of CMV UL54 DNA polymerase were isolated: A885T and N898D.

[e] Genotypic resistance tests were performed using the same blood sample (the second test has been retrospectively assessed).

[f] A "0" entry indicates that no amino acid change was evidenced.

ically active antiviral drug to treat pediatric CMV disease was available in France at that time and (ii) the severe inherited immune deficiency of our patient required a prophylaxis against HSV-1 infection, a VACV-based prophylaxis was implemented.

Nine weeks later, the patient presented with CMV viremia (3.4 $\log_{10}$ copies/ml). A CMV genotypic resistance test showed discrepant results in blood and CSF: whereas a resistant strain still predominated in CSF (H520Q and L545S mutations), a wild-type virus was identified in blood. CMV resistance was retrospectively assessed again in the previously tested blood sample: this second test isolated a resistant strain (H520Q and L545S mutations). GCV-plus-FOS bitherapy was started again but failed to decrease the CMV load in blood or CSF. During subsequent months, CMV genotypic resistance tests were performed in blood samples and showed M460I and L545S mutations in UL97 phosphotransferase and UL54 DNA polymerase, respectively (Table 1).

Because of the absence of available virologically active antiviral drugs and the progressive development of a refractory aplastic anemia, it was decided to proceed to a second HSCT in our patient as an attempt to cure the inherited immune deficiency. Unfortunately, the child died from multivisceral failure 10 days after a second HSCT attempt.

CMV is one of the clinically most significant viral pathogens causing infections in immunocompromised patients, especially in HSCT recipients. In the transplant setting, the emergence of CMV resistance to antivirals, favored by long-term exposure to antiviral drugs and profound immunodeficiency, constitutes an increasing therapeutic challenge (2). CMV infection of the central nervous system (CNS) is rare in HSCT recipients. Fifteen well-documented cases have been reported (2–13). In most of them, the authors described a high viral load in the CSF contrasting with low peripheral blood viral load (2, 9, 13) or low antigenemia (3, 4), suggesting a high level of viral replication in the CNS. In this study, we have observed a temporal and spatial evolution of mutations in the *UL97* and *UL54* genes of CMV in a child with severe inherited immune deficiency who developed a CMV disease refractory to treatment with both GCV and FOS.

The most striking result was the spatial compartmentalization of CMV mutations in this patient. Indeed, whereas the CMV genotypic resistance tests did not reveal any resistance mutation in blood samples in January 2011 and February 2012, indicating a repopulation of the blood compartment by wild-type strains, the patient developed in September 2011 CMV meningoencephalitis due to a multidrug-resistant strain. Two hypotheses could explain these findings. First, the resistant strain isolated in CSF in 2011 could have been recently selected because of the low CNS penetration of the antiviral prophylaxis. At the time of diagnosis of CMV meningoencephalitis, the child had being treated with VACV prophylaxis for 9 months. Few previous retrospective reports supported the idea of selection of mutations conferring GCV/ACV cross-resistance (14–16). Further large prospective studies are needed to accurately assess the risk of selecting CMV mutations in *UL97* and/or *UL54* genes under conditions of ACV/VACV prophylaxis. Second, prolonged exposure to antivirals between 2009 and 2011 (especially during the long episode of uncontrolled viremia during treatment in 2009 to 2010) could have

contributed to the selection of a resistant strain in blood, which might have fuelled the CNS and persisted in this compartment during a long asymptomatic period before a late symptomatic reactivation. No strain with H520Q (*UL97* gene) and L545S (*UL54* gene) mutations in blood between June 2009 and September 2011 has been detected. However, we cannot exclude the possibility that such a mutant virus had been selected several months or years prior to its detection and persisted in blood and/or CSF as a minor viral quasispecies. Whatever the hypothesis, our results show that blood samples may not be representative of the resistance status of the virus isolated in the CNS compartment. Interestingly, previous reports of CMV CNS diseases which compared the CMV genotypic resistance profiles in concomitant CSF and blood samples described similar profiles in the two compartments (6, 11) or viral compartmentalization, with wild-type CMV in CSF samples and resistant CMV in blood and other sites (5, 8, 9, 13). The authors suggested that decreased CMV fitness due to drug resistance mutations located in *UL97* and/or *UL54* genes could explain the predominance of wild-type strains in the CSF, even in cases of previous selection of resistant mutants in blood (17). This is the first report describing the possibility that meningoencephalitis due to resistant CMV can occur even in cases of recent isolation of wild-type strains in blood.

Three patterns of *UL97* and *UL54* mutations were observed in the successive blood specimens: (i) no drug resistance mutations, (ii) M460I/L545S mutations; and (iii) H520Q/L545S mutations. Although the full-length CMV genome was not characterized in our patient, the same natural polymorphisms located in UL97 phosphotransferase (amino acid changes Q19E, N68D, S108N, and I244V) and UL54 DNA polymerase (amino acid changes A885T and N898D) were observed in all strains. Consequently, these viruses may represent different evolution patterns under antiviral pressure of the viral quasispecies derived from a unique virus. Because population-based sequencing could detect only the viral variants present in more than 20% of the total viral population, minor variants might not have been isolated. Indeed, the resistant strain harboring the M460I mutation, which was evidenced in blood in May 2010, was not detected in January 2011 and February 2012. However, this mutation was again detected in blood in May to June 2012, indicating the long-term persistence of this archived resistant strain among the blood quasispecies. Moreover, the retrospective reanalysis of the blood sample collected in February 2012 showed 2 different results of resistance patterns, a wild-type strain in the first case and a virus with H520Q/L545S mutations in the other case, indicating the cocirculation of distinct quasispecies harboring different mutations in blood. Our findings could have a major impact for management of CMV disease. Indeed, this report (i) confirms that analysis of concomitant blood samples cannot predict the genotypic resistance profile of strains in the CSF, (ii) indicates that CMV molecular resistance monitoring should be performed in all body compartments where CMV is present or suspected, (iii) suggests that the initial antiviral treatment of CMV CNS disease should be adapted to all previously identified viral resistance mutations in any body compartment, and (iv) suggests that monitoring of CMV molecular resistance should be performed frequently, especially in cases of long exposure to antiviral drugs, because of the lack of sensitivity of CMV gene direct sequencing for the detection of minor resistant variants.

We highly suspect that the resistance mutations had been selected in our patient during the prolonged uncontrolled viremia in 2009 to 2010. Several factors have been previously reported as being associated with the selection of resistant strains, especially a high CMV load, a prolonged exposure to GCV, and suboptimal plasma GCV levels (2, 18). All of these risk factors, especially the long exposure to antiviral treatments, were present in our patient. Interestingly, dramatically low trough GCV levels were observed, despite the use of the GCV recommended daily doses (i.e., 5 mg/kg/12 h). Although GCV is widely used to treat CMV infection in immunocompromised children, previous pediatric pharmacokinetics reports about GCV exposure are sparse and heterogeneous. Further prospective studies are urgently needed, especially in order (i) to assess that the currently recommended GCV daily doses are appropriate in the pediatric population and (ii) to study whether monitoring of GCV plasma levels should be recommended in children in order to avoid the selection of resistance mutations.

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