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Running title: Horizontal transmission of iciHHV-6A

Abbreviations: CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; FOS, foscarnet; GCV, ganciclovir; GVHD, graft versus host disease; HCMV, Human cytomegalovirus; HHV-6, Human herpesvirus 6; iciHHV-6, inherited chromosomally integrated HHV-6; HSCT, hematopoietic stem cells transplantation; HSV-1, Herpes simplex virus type 1; MMF, mycophenolate mofetil

Abstract

HHV-6A and HHV-6B are found as inherited and chromosomally integrated forms (iciHHV-6A and -6B) into all germinal and somatic cells and vertically transmitted in a Mendelian manner in about 1% of the population. They were occasionally shown to be horizontally transmitted through hematopoietic stem cell transplantation. Here, we present a clinical case of horizontal transmission of iciHHV-6A from donor to recipient through liver transplantation. Molecular analysis performed on three viral genes (7.2 kb) in the recipient and donor samples supports transmission of iciHHV-6A from the graft. Transmission was followed by reactivation, with high viral loads in several compartments. The infection was uncontrollable, leading to severe disease and death, despite antiviral treatments and the absence of resistance mutations. This case highlights the fact that physicians should be aware of the possible horizontal transmission of iciHHV-6 and its consequences in case of reactivation in immunocompromised patients.

Introduction

Human herpesvirus-6A and -6B (HHV-6A and HHV-6B) are widespread with a seroprevalence over 90% in adults. Primary infection is associated with a usually benign skin rash in infants (exanthema subitum), but reactivation from latency can lead to severe disease, such as hepatic, neurological, and disseminated infections (1), especially in immunocompromised patients. Opportunistic HHV-6 infections are quite common in transplant patients, occurring in 20 to 90% of solid organ and 40 to 70% of hematopoietic stem cell recipients, and are increased by immunosuppressive treatments with corticosteroids (2,3,4). Most are HHV-6B and secondary to the reactivation of an endogenous strain of the recipient. However in some cases, it can be due to an exogenous strain either from the graft or the community (4). The drugs initially developed against Human cytomegalovirus (HCMV), i.e. ganciclovir, foscarnet and cidofovir, have been shown to have inhibitory effects against

HHV-6A and HHV-6B in in vitro studies (5). Their clinical use for HCMV reactivation in transplant recipients could concomitantly reduce HHV-6 reactivation. It has been observed in several studies where liver transplant patients on (val)ganciclovir prophylaxis had a lower incidence of HHV-6 infection compared with those who received acyclovir or no prophylaxis (6-8). For the therapy of HHV-6 infections and related symptoms, a few case reports and small patient series suggested an impact of antiviral drugs on the viral load and the resolution of clinical signs but it is not always reliably effective and the success rate was estimated to approximately 60% (3,4,9). Because of the lack of controlled trials proving their benefit, none is officially approved for the prophylaxis, the preemptive therapy and the treatment of HHV-6 infections, except a recommendation in case of encephalitis, and no specific dosage and duration are established. Furthermore, they–exhibit toxicity to the bone marrow or kidneys requiring appropriate use in the clinical context. Importantly, an efficient cellular immunity, possibly restored by a reduction in the degree of immunosuppression, remains essential to control active infections and improve clinical symptoms (9,10).

In parallel to the usual infection, the genome of HHV-6A and -6B is covalently integrated into a chromosome of all germinal and somatic cells in approximately 1% of the world population and is vertically transmitted in a Mendelian manner (iciHHV-6 for inherited chromosomally integrated HHV-6, also called ciHHV-6) (11). The presence of iciHHV-6 is therefore always associated with high viral loads in patient samples, over 5.5 $\log_{10}$ copies per mL of whole blood or at least one genome copy per cell, and can be confounded with a high HHV-6 reactivation leading to unnecessary treatments. Indeed, it was first assumed to be an exclusive latent form, until in vitro and in vivo studies evidenced that viral expression could occur (11). The clinical impact on iciHHV-6 carriers generally appears to be limited, with a statistical association with angina pectoris (12). However, the reactivation of an iciHHV-6A with production of infectious particles associated to severe disease was demonstrated in a child with severe combined immunodeficiency (13). The antiviral drug and prednisolone treatment

improved the clinical symptoms but the patient recovered only after hematopoietic stem cells transplantation (HSCT) and immunological reconstitution.

To date, little is known about the consequences of horizontal transmission of iciHHV-6A or -6B. Prevalence studies showed that about 0.2% and 0.4% of blood donors from North America and 0.2% and 0.9% of European blood donors harbored iciHHV-6A and iciHHV-6B respectively (14). Several cases of HSCT with donor cells containing iciHHV-6A or iciHHV-6B have been described, but without frequent associated symptoms, except an 1.7-fold increase of acute graft versus host disease (GVHD) (15,16,17). Here we present, for the first time, a clinical case of horizontal transmission of iciHHV-6A from donor to recipient through a liver transplantation, followed by reactivation associated with confusion, profuse diarrhea, and finally death, despite antiviral treatment.

Case report

A 53-year-old woman was hospitalized in the intensive care unit with disorientation and profuse diarrhea (> 1L/24h) without fever or symptoms of meningitis. She had undergone hepatic transplantation 23 days before for cirrhosis of mixed etiology (alcoholic and metabolic) in the context of type 2 diabetes complicated by diabetic nephropathy. No antimicrobial prophylaxis was given before transplantation. Basiliximab was used as induction therapy immediately after transplantation and continued for 5 days. Since day 6, iImmunosuppressive therapy consisted of 800 mg cyclosporine, 15 mg prednisolone, and 500 mg mycophenolate mofetil (MMF) daily.

Brain MRI showed no signs of encephalitis, but electroencephalogram provided evidence of encephalopathy at day 34. Cerebrospinal fluid (CSF) analysis showed no signs of meningitis, with 109 red cells/mm^3, 1 leucocyte/mm^3, glycorrhachia of 5.4 mM, glycemia of 5.8 mM, and proteinorrachia of 0.30 g/L. Bacterial cultures, cryptococcal antigen and toxoplasma PCR of

CSF were all negative. In contrast, multiplex herpes PCR (Herpes consensus generic®, Argène Biomérieux), which screens for HSV-1, HSV-2, VZV, HCMV, EBV, and HHV-6, was positive in CSF for HHV-6. HHV-6 and cellular DNA from various tissues were quantified by real-time PCR (HHV6 R-gene®, Argène Biomérieux) (Fig.1). Viral loads ranged from 2.4 to 4.4 $\log_{10}$ copies/10^6 cells in whole blood and 7.4 $\log_{10}$ copies/10^6 cells in CSF (4.5 $\log_{10}$ cop/mL), compatible with an active HHV-6 infection associated with neurological symptoms. Of note, no search for underlying immune deficiencies was performed in the recipient.

Stool samples were negative for enteropathogenic bacteria (*Salmonella sp, Shigella sp, Campylobacter sp, Yersinia sp, Clostridium difficile*) and parasites (*Cryptosporidium sp, Microsporidium sp*). Gastrointestinal biopsies (stomach, small intestine, and colon) were negative for CMV by immunostaining and PCR, but highly positive for HHV-6 by PCR (4.8 – 8.4 $\log_{10}$ copies/10^6 cells) (Fig.1). DNA from saliva and bronchoalveolar lavage were also positive for HHV-6. Anatomopathological examination of a colon biopsy sampled at day 32 showed inflamed mucosa with intranuclear inclusions and multinucleation, consistent with HHV-6 infection (18). Transaminases were not significantly increased until day 96 ($\leq$ 2N). GVHD and acute liver rejection were excluded by liver biopsy at day 32 and post-mortem.

Since HSV-1 and EBV viremia were positive at day 38 (Fig.1), immunosuppressive therapy was reduced. Prednisolone dose was decreased to 10 mg/day, MMF was interrupted and cyclosporine treatment was minimized to target lower blood concentrations than in standard treatments (300 – 400 ng/mL). The patient was treated with foscarnet (6 g/day) from day 35 to day 47 post-transplantation with no effect on viral load (Fig.1), before being discontinued due to severe hypokalemia. It was replaced by ganciclovir (450 mg twice a day) from day 47 to day 62, before pancytopenia led to another switch to foscarnet (6 g/day). Further evolution of the patient's condition was characterized by persistence of pancytopenia, increased disorientation, profuse diarrhea (exudative enteropathy), and several episodes of septic shock.

Last one, at day 96, was due to an ischemic cholangitis (transaminases > 10N). Despite treatment with noradrenaline and antibiotics, the patient died from multiple organ failure 98 days after transplantation.

We quantified the HHV-6 DNA from various compartments of the recipient and donor to identify the source of the HHV-6 infection using a real-time PCR method developed by Gautheret-Dejean (1). Viral loads in the recipient varied from one compartment to another. It was less than 4 $\log_{10}$ cop/10^6 cells in whole blood and negative in the native liver, excluding the presence of iciHHV-6 in the patient. By contrast, the viral loads were 7.5 and 6.9 $\log_{10}$ copies/10^6 cells in the liver and spleen of the donor, respectively, suggesting the presence of iciHHV-6. Reactivation could either have come from an endogenous strain or that present in the transplanted liver, since the patient was seropositive for HHV-6 before transplantation (titer 1:160; IgG HHV-6 IFA Biotrin, Diasorin). We explored these possibilities by sequencing the HHV-6 U39 gene from various compartments: donor spleen, transplanted liver, and colon biopsy, CSF, and saliva of the recipient (19). This gene was chosen for its variability (6.2% of nucleotide divergence). The same HHV-6A sequence was found in all donor and recipient samples, characterized by 17 nucleotide differences from the U1102 reference strain (GenBank accession number NC_001664.2). It was identical to the iciHHV-6A LEI_1501 strain from UK (KT355575) (20) and 6 others iciHHV-6A studied in our laboratory, that illustrates low diversity among iciHHV-6A strains. Nevertheless, it was different from the sequences of 41 HHV-6A or iciHHV-6A strains studied in our laboratory or elsewhere, including GS (KC465951) and AJ (KP257584) strains (21-24). These results support horizontal transmission of iciHHV-6A present in the transplanted liver from the donor to the recipient, followed by reactivation of this strain in various tissues.

As antiviral treatments with ganciclovir or foscarnet failed to decrease viral loads and improve clinical symptoms, resistance mutations were searched in the relevant viral genes, U38 (DNA polymerase) and U69 (phosphotransferase). We sequenced DNA from the donor

spleen, the transplanted liver, a colon biopsy, and saliva from the recipient before (day 32) and after (day 90) treatment. The sequences were identical in all samples and identical to other iciHHV-6A strains, including LEI_1501 (20). We found four amino acid changes (R148K and F497Y in U38, E67G and N119D in U69) also found in HHV-6A or HHV-6B strains proven to be sensitive to ganciclovir and foscarnet (25). The remaining eight changes (A500V, M997V in U38; D298G, V300I, G4A, I51N, L361F and E522K in U69) are not known to be resistance mutations, suggesting that this iciHHV-6A strain was sensitive to antiviral drugs.

Overall, the sequence identity between three genes (7.2 kb) of the donor and recipient samples strengthens the hypothesis of transmission of iciHHV-6A from the graft. The viral loads quantified in various compartments including saliva, CSF and gastrointestinal tract were consistent with strong reactivation of the iciHHV-6A present in the transplanted liver. Increased allograft rejection among patients with HHV-6 reactivation in the transplanted organ have been described, resulting from boosted immune system and leucocytes infiltration or syncytial giant cell hepatitis (1,4,26). Such specific histopathology was not observed here, neither were signs of acute liver rejection. The most likely cause for fatal outcome here is the reactivation of iciHHV-6 from the graft and infection of the brain, digestive tract and saliva. This was associated with encephalopathy, profuse diarrhea, and followed by a series of septic shocks which lead to multiple organ failures and death. One can assume that the infection was uncontrolled due to several reasons: (i) the absence of antimicrobial prophylaxis and the maintenance of corticosteroid treatment could have promoted this reactivation, (ii) pharmacokinetics of antiviral drugs were not evaluated in the recipient and intracellular concentrations may have been insufficient to completely inhibit replication, even of a sensitive strain, (iii) an immune deficiency or poor reconstitution in the recipient could have restricted the role of anti-HHV-6 cellular immunity, especially in a context where each cell of

the transplant is a limitless source of reactivation. This is the first description of iciHHV-6A reactivation from a transplanted solid organ associated with clinical disease. Physicians and transplant surgeons should be aware of the possibility of horizontal transmission of iciHHV-6 and its consequences in case of reactivation, since approximately 1% of donors harbor iciHHV-6.

References

1. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and Clinical Aspects of Human Herpesvirus 6 Infections. Clin Microbiol Rev 2015; 28(2): 313–335.

2. Le J, Gantt S, the AST Infectious Diseases Community of Practice. Human Herpesvirus 6, 7 and 8 in Solid Organ Transplantation: HHV-6, 7 and 8 in SOT. Am J Transplant 2013; 13(s4): 128–137.

3. Ljungman P, Singh N. Human herpesvirus-6 infection in solid organ and stem cell transplant recipients. J Clin Virol 2006; 37 Suppl 1: S87-91.

4. Phan TL, Lautenschlager I, Razonable RR, Munoz FM. HHV-6 in liver transplantation: A literature review. Liver Int 2017; 26.

5. De Clercq E, Naesens L. In search of effective anti-HHV-6 agents. J Clin Virol 2006; 37 Suppl. 1: S82-S86.

6. Humar A, Kumar D, Caliendo AM, Moussa G, Ashi-Sulaiman A, Levy G et al. Clinical impact of human herpesvirus 6 after liver transplantation. Transplantation 2002; 73: 599-604.

7. Humar A, Washburn K, Freeman R, Paya CV, Mouas H, Alecock E et al. An assessment of interactions between hepatitis C virus and herpesvirus reactivation in liver transplant recipients using molecular surveillance. Liver Transpl 2007; 13: 1422-1427.

8. Ohashi M, Sugata K, Ihira M, Asano Y, Egawa H, Takada Y et al. Human herpesvirus 6 infection in adult living related liver transplant recipients. Liver Transpl 2008; 14: 100-109.

9. Vinnard C, Barton T, Jerud E, Blumberg E. A report of human herpesvirus 6-associated encephalitis in a solid organ transplant recipient and a review of previously published cases. Liver Transpl 2009; 15(10): 1242-6.

10. Admiraal R, de Koning CCH, Lindemans CA, Bierings MB, Wensing AMJ, Versluys AB et al. Viral reactivations and associated outcomes in the context of immune reconstitution after pediatric hematopoietic cell transplantation. J Allergy Clin Immunol 2017; 7.

11. Pellett PE, Ablashi DV, Ambros PF, Agut H, Caserta MT, Descamps V, et al. Chromosomally integrated human herpesvirus 6: questions and answers. Rev Med Virol 2012; 22(3): 144–155.

12. Gravel A, Dubuc I, Morissette G, Sedlak RH, Jerome KR, Flamand L. Inherited chromosomally integrated human herpesvirus 6 as a predisposing risk factor for the development of angina pectoris. Proc Natl Acad Sci U S A. 2015; 112(26): 8058-63.

13. Endo A, Watanabe K, Ohye T, Suzuki K, Matsubara T, Shimizu N, et al. Molecular and Virological Evidence of Viral Activation From Chromosomally Integrated Human Herpesvirus 6A in a Patient With X-Linked Severe Combined Immunodeficiency. Clin Infect Dis 2014; 59(4): 545–548.

14. Tweedy J, Spyrou MA, Pearson M, Lassner D, Kuhl U, Gompels UA. Complete Genome Sequence of Germline Chromosomally Integrated Human Herpesvirus 6A and Analyses Integration Sites Define a New Human Endogenous Virus with Potential to Reactivate as an Emerging Infection. Viruses 2016; 8(1).

15. Jeulin H, Salmon A, Gautheret-Dejean A, Agut H, Bordigoni P, Fortier B et al. Contribution of human herpesvirus 6 (HHV-6) viral load in whole blood and serum to investigate integrated HHV-6 transmission after haematopoietic stem cell transplantation. J Clin Virol 2009; 45(1): 43-6.

16. Miura H, Kawamura Y, Kudo K, Ihira M, Ohye T, Kurahashi H, et al. Virological analysis of inherited chromosomally integrated human herpesvirus-6 in three hematopoietic stem cell transplant patients. Transpl Infect Dis 2015; 17(5): 728–731.

17. Hill JA, Magaret AS, Hall-Sedlak R, Mikhaylova A, Huang ML, Sandmaier BM, et al. Outcomes of hematopoietic cell transplantation using donors or recipients with inherited chromosomally integrated HHV-6. Blood. 2017; 130(8): 1062-1069.

18. Roux J, Battistella M, Fornecker L, Legoff J, Deau B, Houhou N, et al. Human Herpesvirus-6 cytopathic inclusions: an exceptional and recognizable finding on skin biopsy during HHV6 reactivation after autologous stem-cell transplantation. Am J Dermatopathol 2012; 34(6): e73–76.

19. Achour A, Malet I, Le Gal F, Dehée A, Gautheret-Dejean A, Bonnafous P, et al. Variability of gB and gH genes of human herpesvirus-6 among clinical specimens. J Med Virol 2008; 80(7): 1211–1221.

20. Zhang E, Cotton VE, Hidalgo-Bravo A, Huang Y, Bell AJ, Jarrett RF, et al. HHV-8-unrelated primary effusion-like lymphoma associated with clonal loss of inherited

chromosomally-integrated human herpesvirus-6A from the telomere of chromosome 19q. Sci Rep 2016; 6: 22730.

21. Gravel A, Ablashi D, Flamand L. Complete Genome Sequence of Early Passaged Human Herpesvirus 6A (GS Strain) Isolated from North America. Genome Announc 2013; 1(3).

22. Gravel A, Hall CB, Flamand L. Sequence analysis of transplacentally acquired human herpesvirus 6 DNA is consistent with transmission of a chromosomally integrated reactivated virus. J Infect Dis 2013; 207(10): 1585–1589.

23. Hill JA, Sedlak RH, Zerr DM, Huang ML, Yeung C, Myerson D, et al. Prevalence of chromosomally integrated human herpesvirus 6 in patients with human herpesvirus 6-central nervous system dysfunction. Biol Blood Marrow Transplant 2015; 21(2): 371–373.

24. Tweedy J, Spyrou MA, Donaldson CD, Depledge D, Breuer J, Gompels UA. Complete Genome Sequence of the Human Herpesvirus 6A Strain AJ from Africa Resembles Strain GS from North America. Genome Announc 2015; 3(1).

25. Manichanh C, Grenot P, Gautheret-Dejean A, Debré P, Huraux JM, Agut H. Susceptibility of human herpesvirus 6 to antiviral compounds by flow cytometry analysis. Cytometry 2000; 40(2): 135–140.

26. Potenza L, Luppi M, Barozzi P, Rossi G, Cocchi S, Codeluppi M, et al. HHV-6A in syncytial giant-cell hepatitis. N Engl J Med 2008; 359(6): 593-602.

Legend of the figure

Figure 1: Clinical and biological course of HHV-6 infection in various compartments after liver transplantation.

FOS, foscarnet; GCV, ganciclovir; MMF, mycophenolate mofetil; HSV-1, Herpes simplex virus type 1; EBV, Epstein-Barr virus.