Journal of Microbiology, Immunology and Infection (2014) 47, 418–421

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Incidence of human herpesvirus 6 in clinical samples from Swedish patients with demyelinating diseases

Rasmus Gustafsson*, Renate Reitsma, Annelie Strålfors, Andreas Lindholm, Rayomand Press, Anna Fogdell-Hahn

Department of Clinical Neuroscience, The Multiple Sclerosis Research Group, Karolinska Institutet, Stockholm, Sweden

Received 6 January 2012; received in revised form 8 March 2013; accepted 27 March 2013
Available online 31 May 2013

KEYWORDS
CIDP;
Guillain–Barré syndrome;
HHV-6;
IFNβ;
Multiple sclerosis;
NAb

Background: Human herpesvirus 6 (HHV-6) has been reported to be associated with multiple sclerosis (MS) and Guillain–Barré syndrome (GBS).
Methods: We analyzed cell-free HHV-6 DNA as an indication of active infection in the peripheral blood and cerebrospinal fluid (CSF) of Swedish patients with GBS, patients with chronic inflammatory demyelinating polyradiculoneuropathy, treatment-naïve patients with possible MS, interferon-β treated MS patients [with or without neutralizing antibodies (NAbs)], and control patients with headache.
Results: One of 14 GBS patients and one of eight patients with chronic inflammatory demyelinating polyradiculoneuropathy were positive for HHV-6 DNA in serum. Of the 27 treatment-naïve possible MS patients, two were positive in plasma and one in CSF. HHV-6 DNA was detected in the serum of three of 79 NAb+ patients and one of 102 NAb-interferon-β treated MS patients. HHV-6 DNA could not be detected in the plasma or CSF of any of the 33 controls, although the differences were not statistically significant.
Conclusion: Our results do not suggest active HHV-6 infection to be a common phenomenon in any of the patient groups studied.

* Corresponding author. Department of Clinical Neuroscience, The Multiple Sclerosis Research Group, CMM L8:00, SE-171 76 Stockholm, Sweden.
E-mail address: Rasmus.Gustafsson@ki.se (R. Gustafsson).

http://dx.doi.org/10.1016/j.jmii.2013.03.009

Introduction

Multiple sclerosis (MS), Guillain–Barré syndrome (GBS), and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are diseases characterized by autoimmune demyelination of the nervous system. The mechanisms behind the triggering of these diseases remain obscure, but viral infections are possible candidates. Human herpesvirus 6 (HHV-6) is a ubiquitous virus[1] that has been suggested to be associated with MS[2,3] and GBS[4] but has not yet been investigated in CIDP. Furthermore, relapse risk has been shown to positively correlate with HHV-6 viral load[5] and anti-HHV-6 IgG titers.[6]

Interferon-β (IFNβ) is a first-line treatment of relapsing–remitting MS but also an important antiviral cytokine. Treatment with IFNβ has been shown to reduce the HHV-6A DNA load in sera of patients with MS.[7] Because about 20% of patients treated with IFNβ develop neutralizing antibodies (NAbs) against the drug, thereby blocking the efficacy of the treatment,[8] and possibly an antiviral systemic effect, a difference regarding the level of viral load between NAb positive and NAb negative patients might be detected in serum from these patients.

It has been difficult to establish a causal relationship between HHV-6 and neurological diseases. A proof of principle would be if one could observe a clinical improvement after antiviral treatment targeting HHV-6 in patients with neuroinflammatory diseases, who—during the course of disease—have shown evidence of active viral infection. However, this would require a robust method to screen for candidate patients eligible for such a trial. Hence, we aimed to determine the frequency of HHV-6 DNA in plasma, cerebrospinal fluid (CSF), and serum samples of patients with GBS, CIDP or possible MS in a clinical setting, using samples taken as standard clinical practice. In addition, we investigated whether reduced responsiveness to IFNβ, due to NAbs, leads to increased detection of HHV-6 cell free DNA.

Materials and methods

We analyzed serum and CSF samples from 14 patients with GBS (Asbury criteria) and eight patients with CIDP (EFNS 2005 criteria). Furthermore, plasma and CSF samples from 27 treatment-naïve patients who were newly diagnosed or showed signs of MS but had not yet fulfilled the full diagnostic criteria for MS were analyzed.[9] This group is hereafter referred to as possible MS. As controls, serum and CSF samples from 33 headache patients investigated for noncomplicated headache were analyzed. GBS and CIDP are demyelinating diseases of the peripheral nervous system (reviewed by Hughes and Cornblath[10]). GBS was diagnosed by the combination of typical clinical features of ascending paralysis and areflexia during a progressive phase lasting a maximum of 4 weeks, in addition to features of a peripheral demyelinating neuropathy on neurophysiological examination.[11] CIDP was characterized by a subacute progressive proximal and distal paralysis of the extremities with a progressive phase lasting a minimum of 8 weeks and neurophysiological features similar to those of GBS.[12] In the GBS group one patient was treated with intravenous methyl-prednisone and one with intravenous immunoglobulin, and in the CIDP group two patients were treated with intravenous immunoglobulin and one with oral prednisone and azathioprine before collection of blood and CSF. The remainder of the patients with GBS and CIDP were still treatment-naïve at the time-point of blood and CSF collection. MS is a demyelinating disease of the central nervous system (reviewed by Hafler et al[13]) where detection of dissemination of lesions is separated in time and space as revealed by clinical symptoms or, for example, magnetic resonance imaging.[9] We also analyzed serum samples from patients with MS treated with IFNβ, 79 patients with high titer NAbs (NAb+) (>1280 ten-fold reduction units (TRU)/ml), and 102 patients without NAbs (NAb−) (<10 TRU/ml) (Table 1), as determined using the myxovirus resistance protein A (*MxA*) gene expression assay previously described.[14] All samples were frozen directly after collection and stored at −80°C until analysis. The study was approved by the National Ethics Committee of Sweden (Ö-32-2005).

DNA extraction

Viral DNA was extracted from 560 μL plasma or serum and CSF from patients with possible MS and controls with headache. From patients with GBS and CIDP, DNA was extracted from 200 μL serum and CSF using a commercial kit (Qiagen, GmbH, Hilden, Germany). For contamination control, water was added to every fifth column. DNA was eluted in 100 μL buffer for the possible MS and headache patients and in 80 μL for the GBS and CIDP patients and stored at −80°C.

Nested polymerase chain reaction

All samples were screened for HHV-6 DNA using a nested polymerase chain reaction (PCR) previously described.[15] Tests were considered valid if all water controls remained negative and if the viral laboratory strains U1102 and Z29, used as positive controls, were amplified. To discriminate between HHV-6A and 6B, in the virus positive serum samples of NAb+ or NAb− MS patients, a variant-specific nested PCR targeting the immediate early gene was performed as previously described.[16] The MCP nested PCR sensitivity was 240 copies/mL, as determined by real-time quantitative PCR as previously described.[17] Samples from IFNβ treated patients were run in duplicates.

Statistical analysis

Fisher's exact test ($\alpha < 0.05$, two-sided) was used for statistical calculations using the software GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA).

Results

HHV-6 in possible MS, GBS, and CIDP plasma, serum, or CSF samples

HHV-6 DNA was found in 3 of the 27 patients with possible MS (2 in plasma and 1 in the CSF), as well as one of 14

Table 1 Presence of HHV-6 DNA in plasma, serum, or CSF of patients with possible MS, GBS, or CIDP, or headache controls; and in serum of IFNβ treated MS patients positive (+) or negative (−) for NAbs against therapeutic IFNβ

Diagnosis	Number of patients	Females	Median age	Sample type	% HHV-6 positive	HHV-6 subtype	*p*
MS[a]	27	25	49	Plasma	7.4 (2/27)	NT	0.20, 0.18,[b] 0.11[c]
				CSF	3.7 (1/27)	NT	0.45
GBS	14	8	65	Serum	7.1 (1/14)	NT	0.30
				CSF	0 (0/14)	−	N/A
CIDP	8	2	56	Serum	12.5 (1/8)	NT	0.20
				CSF	0 (0/8)	−	N/A
Headache	33	26	45	Plasma	0 (0/33)	−	−
				CSF	0 (0/33)	−	−
MS[d] NAb+	79	56	48	Serum	3.8 (3/79)	2*A, 1*B	0.32
MS[d] NAb−	102	80	46	Serum	1.0 (1/102)	1*A	−

[a] Treatment-naïve patients with possible MS.
[b] Compared to IFNβ MS patients.
[c] Compared to NAb− IFNβ MS patients.
[d] IFNβ treated MS patients positive (+) or negative (−) for NAbs against the therapeutic IFNβ.
Treatment-naïve MS, GBS, and CIDP patients are compared with headache controls, if other comparisons are not specified. NAb+ MS patients are compared with NAb− patients.
N/A = not applicable; NT = not tested.

GBS and one of eight CIDP serum samples. None of the patients with possible MS were positive in both plasma and CSF. The single HHV-6 positive patient with GBS was undergoing methyl-prednisone treatment at the time-point of blood and CSF collection. None of the 33 control patients with headache were HHV-6 positive (Table 1). Compared to the control headache patient group, HHV-6 DNA was not detected more often in possible MS plasma ($p = 0.20$), CSF ($p = 0.45$) or plasma together with CSF ($p = 0.09$), or in GBS ($p = 0.30$) or CIDP ($p = 0.20$) serum samples. None of the HHV-6 positive patients with possible MS were in clinical relapse. However, the HHV-6 positive GBS and CIDP patients were in relapse at the point of sampling.

HHV-6 in MS NAb+ or NAb− patients

Clinical serum samples from NAb+ and NAb− MS patients (Table 1) were analyzed, and DNA was found in three of 79 NAb+ and one of 102 NAb− patients, which was not statistically significant ($p = 0.32$). A virus-specific nested PCR revealed that all positive samples in the IFNβ treated group were HHV-6A, except one in the NAb+ group, which was HHV-6B (Table 1). Furthermore, no difference was detected in the whole group of IFNβ treated patients ($p = 0.18$), or between the NAb− subgroup ($p = 0.11$) and treatment-naïve patients with possible MS. The NAb− subgroup was chosen for further analysis as the IFNβ treatment is not impaired by circulating NAbs. Among the HHV-6 positive patients, one of the NAb+ patients was in relapse, whereas the others were not.

Discussion

HHV-6 has been associated with MS[2] and GBS.[4] Our aim was to replicate these findings in cross-sectional samples collected as part of routine clinical practice. If HHV-6 infection can be consistently identified in patients' serum or CSF of patients with MS and GBS during active phases of the respective diseases, treatment with antiviral therapy targeting HHV-6 may have a positive influence on the outcome of MS and GBS. A first step to test this hypothesis is the capability to easily identify patients who have HHV-6 virions or anti-HHV-6 responses in serum or plasma and CSF. Several serological methods, such as enzyme-linked immunosorbent assay targeting antiviral IgG or IgM, or molecular methods, such as real-time quantitative PCR, could be used for this purpose, but nested PCR ought to be one of the most sensitive, rapid, and cost-effective methods and was therefore used in this study. Other researchers have shown that treatment with rhIFNβ reduces the titer of HHV-6 DNA in serum,[7] and thus, this effect might not be seen in MS patients treated with rhIFNβ who have developed NAbs against the drug.

We did not find HHV-6 infection in a significantly higher number of patients with GBS, CIDP, or MS, nor did we observe any difference between IFNβ treated and treatment-naïve MS patients that would allow us to confirm the association noted in previous reports.[2,4,5] Of the treatment-naïve patients with possible MS who tested positive, none had detectable levels of HHV-6 virus in both CSF and plasma, indicating a local infection, not passing over the blood—brain barrier. Screening for HHV-6 DNA in a cross-sectional setup at one time point only might be a too stringent approach to identify patients with active HHV-6 infection. Following patients by longitudinal sampling over the course of the disease might be a better approach if the aim is to detect HHV-6 as a possible agent contributing to the pathogenesis of the disease. The limited number of patients with active infection found of this study makes it difficult to state that HHV-6 infection plays a major causative role in the pathogenesis of MS, GBS, and CIDP.

Conflicts of interest

R. Gustafsson, R. Reitsma, A. Strålfors, A. Lindholm, and R. Press report no conflicts of interest. Dr. Fogdell-Hahn reports receiving research grants from Biogen Idec, Sanofi-Aventis and Merck Serono.

Acknowledgments

This study was supported by the Board of Doctoral Education at Karolinska Institutet. We thank Malin Lundkvist and Ingegerd Löfving-Arvholm for technical assistance.

References

1. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986; **234**:596—601.
2. Soldan SS, Berti R, Salem N, Secchiero P, Flamand L, Calabresi PA, et al. Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nat Med* 1997;**3**:1394—7.
3. Goodman AD, Mock DJ, Powers JM, Baker JV, Blumberg BM. Human herpesvirus 6 genome and antigen in acute multiple sclerosis lesions. *J Infect Dis* 2003;**187**:1365—76.
4. Galvan M, Rotola A, Govoni V, Granieri E, Cassai E, Di Luca D. Simultaneous Guillain—Barre syndrome and active human herpesvirus 6 infection in the central nervous system. *J Clin Virol* 2007;**38**:271—2.
5. Alvarez-Lafuente R, De las Heras V, Bartolomé M, Picazo JJ, Arroyo R. Relapsing—remitting multiple sclerosis and human herpesvirus 6 active infection. *Arch Neurol* 2004;**61**:1523—7.
6. Simpson Jr S, Taylor B, Dwyer DE, Taylor J, Blizzard L, Ponsonby AL, et al. Anti-HHV-6 IgG titer significantly predicts subsequent relapse risk in multiple sclerosis. *Mult Scler* 2012; **18**:799—806.
7. Alvarez-Lafuente R, De Las Heras V, Bartolomé M, Picazo JJ, Arroyo R. Beta-interferon treatment reduces human herpesvirus-6 viral load in multiple sclerosis relapses but not in remission. *Eur Neurol* 2004;**52**:87—91.
8. Sominanda A, Hillert J, Fogdell-Hahn A. In vivo bioactivity of interferon beta in multiple sclerosis patients with neutralizing antibodies is titer dependent. *J Neurol Neurosurg Psychiatry* 2008;**79**:57—62.
9. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;**50**: 121—7.
10. Hughes RA, Cornblath DR. Guillain—Barre syndrome. *Lancet* 2005;**366**:1653—66.
11. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain—Barre syndrome. *Ann Neurol* 1990;**27**: S21—4.
12. Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP). Report from an Ad Hoc Subcommittee of the American Academy of Neurology AIDS Task Force. *Neurology* 1991;**41**:617—8.
13. Hafler DA, Slavik JM, Anderson DE, O'Connor KC, De Jager P, Baecher-Allan C. Multiple sclerosis. *Immunol Rev* 2005;**204**: 208—31.
14. Bertolotto A, Sala A, Caldano M, Capobianco M, Malucchi S, Marnetto F, et al. Development and validation of a real time PCR-based bioassay for quantification of neutralizing antibodies against human interferon-beta. *J Immunol Methods* 2007;**321**:19—31.
15. Secchiero P, Carrigan DR, Asano Y, Benedetti L, Crowley RW, Komaroff AL, et al. Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis* 1995;**171**: 273—80.
16. Yalcin S, Karpuzoglu T, Suleymanlar G, Mutlu G, Mukai T, Yamamoto T, et al. Human herpesvirus 6 and human herpesvirus 7 infections in renal transplant recipients and healthy adults in Turkey. *Arch Virol* 1994;**136**:183—90.
17. Nitsche A, Müller CW, Radonic A, Landt O, Ellerbrok H, Pauli G, et al. Human herpesvirus 6A DNA is detected frequently in plasma but rarely in peripheral blood leukocytes of patients after bone marrow transplantation. *J Infect Dis* 2001;**183**: 130—3.