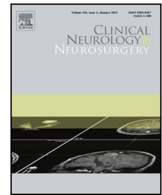
Clinical Neurology and Neurosurgery 119 (2014) 44–48

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro



The participation of varicella zoster virus in relapses of multiple sclerosis

Julio Sotelo*, Graciela Ordoñez, Benjamín Pineda, José Flores

Neuroimmunology Unit, National Institute of Neurology and Neurosurgery of Mexico, Insurgentes Sur 3877, Mexico City 14269, Mexico

ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form
18 December 2013
Accepted 25 December 2013
Available online 10 January 2014

Keywords:
Varicella zoster virus
Multiple sclerosis
Virus and multiple sclerosis
Fingolimod
Natalizumab

ABSTRACT

Objective: Recent studies have documented the apparent participation of varicella zoster virus (VZV) in the etiopathogenesis of multiple sclerosis (MS). The present study aimed to corroborate the possible presence of VZV during exacerbations of MS.
Design: Fifty-three patients with definite MS were included; of them, 31 were studied during the first week of a clinical relapse, whereas 16 were studied during remission; 6 patients with progressive MS were also studied. Genes from 5 herpes viruses: varicella zoster, herpes simplex 1 and 2, Epstein–Barr and herpes 6 were studied by polymerase chain reaction in cerebrospinal fluid and in peripheral blood mononuclear cells (PBMC). As controls 21 patients with inflammatory or functional neurological disorders were included.
Results: DNA from varicella zoster virus was found in the CSF from all MS patients studied during relapse (100%) and in the PBMC from 28 of them (90%). However, VZV DNA was found in the CSF only in 5 MS patients studied during remission (31%) and in the PBMC from 3 of them (19%). VZV DNA was also found, but in lower amounts, in the CSF (83%) and PBMC (33%) from patients with progressive MS. In contrast, VZV was not found either in CSF or in PBMC from controls. Results from the other herpes viruses tested were similar in MS patients and in controls.
Conclusions: Our results corroborate the conspicuous, but ephemeral presence of VZV during relapses of MS and support the idea of VZV involvement in the etiopathogenesis of MS. Recent epidemiological and molecular studies as well as reports of severe VZV infections triggered by specifically induced immunosuppression during therapy of MS give additional support to this potential association.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

During exacerbations of multiple sclerosis (MS) varicella zoster virus (VZV) has been found within blood lymphocytes of MS patients [1,2], the initial report was received with scepticism because VZV had been occasionally mentioned as a potential participant in MS for more than 40 years, but diverse studies along that period had given conflicting results. Most prior investigations were based on the search for antibodies against VZV [3–5]. Even in late reports, with molecular technology, the search for viral DNA had given uncertain results and reviews on the subject concluded that despite a long time and several surveys there was not reliable evidence on the participation of VZV in the etiopathogenesis of MS [6,7]. Nevertheless, in our initial investigations a rather peculiar phenomenon was documented, we found large amounts of VZV DNA inside blood lymphocytes from some MS patients, but this finding was restricted almost exclusively to patients studied within the first few days of an acute relapse of MS; in that study patients in this condition gave unequivocal results on the actual bearing of viral DNA within circulating peripheral blood mononuclear cells (PBMC) [1,8,9]. In contrast, most MS patients studied during the long periods of remission gave negative results, rather similar to the results obtained in healthy controls and in neurological patients with a comprehensive variety of inflammatory and degenerative ailments [8]. These initial results prompted us to conclude either that VZV had an active participation as triggering factor of MS relapses or that our findings corresponded to a non-specific epiphenomenon of viral reactivation associated, but not related, to the clinical relapse of MS [1,8]. The later possibility could be due either to the immunological changes that occur during the immunopathology of active MS or to the intense immunosuppressive treatment usually administered to MS patients at the time of an acute relapse; in this hypothetical situation, a latent VZV could

* Corresponding author at: Insurgentes Sur 3877, 14269 Mexico City, Mexico. Tel.: +52 55 5528 39 48; fax: +52 55 56 06 40 40.
E-mail address: jsotelo@unam.mx (J. Sotelo).

0303-8467/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.clineuro.2013.12.020

Table 1
Oligonucleotide primers.

Viral target	Gene	Forward primer	Reverse primer	TaqMan probe (T)	Product size (pb)
VZV	ORF-31	5′-CACAAAAACACCCGACTCGAA-3′	5′-AAT GGC ACG AAC TCA ACT G-3′	1299	65
HSV-2	gD	5′-AAGATGGCCGATCCCAATC-3′	5′-GTCGGTCAGCTGGTCCAAA-3′	123	165
HSV-1	gD	5′-TGCGGAATTGTGTACTGGAT-3′	5′-GAGGCGTATGCGCTTTG-3′	1093	59
EBV	gp85	5′-TCCGGCAGGTCCTTCGT-3′	5′-CGAGTGACCGAGAAGGGAGAT-3′	1523	67
HHV-6	U29	5′-TTGTCTGTTGTCATGCGTCA-3′	5′-TCCCATACTGGAGCTTTGCT-3′	713	181

initiate replication and be ingested by PBMC as an epiphenomenon without direct participation of the virus in the immunopathogenesis of MS [9].

Further studies in our laboratory indicated that the presence of VZV DNA during relapses of MS was indeed related with the exacerbations of the disorder and not with a non-related epiphenomenon of viral reactivation [10]; in a large group of non-MS patients with autoimmune or with proliferative disorders who were treated with immunosuppressive drugs no viral DNA from VZV could be detected [9,10], whereas in a large group of MS patients we corroborated the brief presence of VZV DNA, usually restricted to the first 10 days of an acute relapse. Disappearing soon afterwards. Moreover, we also found viral particles similar to VZV and large amounts of viral DNA in the cerebrospinal fluid (CSF) from MS patients taken during relapse [11]. These findings vanished from the CSF in the same MS patient when the sample was taken later, during remission of the disease [11,12]. We also found VZV DNA in the PBMC of a significant number of patients with chronic, progressive MS; although the amount of viral DNA in these cases was considerably minor than that found during relapse in MS patients with the relapse/remission form of MS [12,13]. In contrast, in all our studies no similar phenomenon has been observed when other herpes viruses (herpes simplex (HSV), Epstein–Barr (EBV) and human herpes 6 virus (HHV6) were studied in the same group of MS patients, either during relapse or during remission [1,9–11,14].

The purpose of the present study was to corroborate the possible presence of VZV DNA in PBMC and in CSF from a large group of MS patients studied after our initial reports.

2. Patients and methods

Fifty-three patients with diagnosis of definite MS were included; of them, 31 were studied within the first 10 days of an acute relapse, while 16 were studied during remission, at least 3 months after the last exacerbation of the disease; additionally, 6 patients with progressive MS were also included. As controls, 21 neurological patients with vascular or inflammatory disorders were included. No subject included in the study had been vaccinated during infancy against VZV. All patients were Mexican mestizos [2].

2.1. *Molecular determinations of DNA from herpes viruses*

CSF and peripheral blood samples were obtained the same day. The presence of DNA from VZV was searched and quantified (absolute quantification) in PBMC and in CSF by real-time polymerase chain reaction (PCR). Four milliliters of peripheral blood was mixed with 7.2 mg EDTA. Mononuclear cells were separated by gradient centrifugation with Ficoll, and DNA was extracted with Gentra Puregene blood kit (Qiagen); the DNA was dried at room temperature and dissolved in 100 μl sterile water. CSF was obtained by lumbar puncture; 0.5 ml CSF was centrifuged at 2800 × *g* for 40 min to discard cell debris. The supernatant was ultracentrifuged at 70,000 × *g* for 2 h, the pellet was obtained, and DNA extraction was done by Gentra Puregene body fluid kit (Qiagen). In addition, DNA from other herpes viruses that have been implicated in MS was searched (Table 1) (relative quantification) by real-time PCR according with the following protocol: The absolute quantification of viral load was made by real-time PCR. The primers and probes (Table 1) were derived from: VZV, strain ORF 31 (gene gB); herpes simplex 1 (gene gD); herpes simplex 2 (gene gD); Epstein–Barr virus (gene gp85) and human herpes virus 6 (gene U29) designed using Primer Express Software (Ver. 2.0) and synthesized by Applied Biosystems (Mexico). All nucleotides selected had no analogy with other herpes virus, as searched in BLAST (basic local alignment search tool). Each 25 μl of PCR mixture contained 100 ng of DNA either from blood mononuclear cells or from CSF in 5 μl of distilled water, plus 12.5 μl of TaqMan universal PCR master mix (Applied Biosystems), 6.25 μl sterile water and 1.25 μl of primer mixture with final concentrations of each primer of 0.9 μM and of each probe sequence of 0.25 μM. The TaqMan RNAse P was performed to normalize each sample. The standard curve was made by mixing serial 10 fold dilutions of plasmids bearing the gene gB; the gene contained in the pET vector was used. The insert in the standard (pET) was fully sequenced with the CEQ System (Ver. 9.0.25). In addition, reaction mixtures containing the appropriate probe and primer system but without DNA were run on all plates by triplicate and used as negative controls. The PCR mixtures in 96-well microtiter plates were first incubated at 95 °C for 10 min, followed by 50 two-step cycles at 95 °C for 10 s and at 60 °C for 1 min, using an ABI PRISM 7500 real-time PCR system (Applied Biosystems). Each sample was run by triplicate. For each reaction, real-time fluorescence values were measured as a function of the quantity of a reporter dye (6-carboxy-fluorescein [FAM]) released during amplifications. A threshold cycle (Ct) value for each sample was determined as the number of the first cycle at which the measured fluorescence exceeded the threshold limit (10 times the standard deviation of the baseline). Ct values observed for DNA samples were used to calculate the VZV concentration based on the standard curve for the plasmid-containing test sequence. The mass of a single plasmid molecule was determined by the formula: $m = (n)\ (1.096 \times 10^{-21}$ g/bp), where: n = DNA size (bp), m = mass, Avogadro's number = 6.023×10^{23} mol/1 mol, and molecular weight of a double-stranded DNA molecule = 660 g/mol. No cross-reactivity was observed between any of the viral DNA assays performed.

All PCR determinations were run with TaqMan probes; sensibility of real-time PCR for the gB gene from VZV is of 5 viral particles with an R^2 standard value of 0.9936.

The study was approved by the Committee of the National Institute of Neurology and Neurosurgery and written informed consent was obtained by the participants. Statistical analysis was made by chi square for categorical variables; data with parametric distribution were analyzed by ANOVA followed by Tukey, non parametric data were analyzed by Kruskal–Wallis test. Significance value was $p < 0.05$, SPSS version 17 was used.

3. Results

A high content of VZV DNA was present in PBMC as well as in CSF from most MS patients studied during clinical relapse; all 31 patients were positive for VZV DNA in CSF, while 28 (90%) were

Download English Version:

https://daneshyari.com/en/article/6006485

Download Persian Version:

https://daneshyari.com/article/6006485

Daneshyari.com