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ORIGINAL COMMUNICATION

Julio Sotelo
Graciela Ordoñez
Benjamin Pineda

Varicella-zoster virus at relapses of multiple sclerosis

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J. Sotelo (✉) · G. Ordoñez · B. Pineda
Neuroimmunology Unit
National Institute of Neurology and Neurosurgery of Mexico
Insurgentes Sur 3877
14269 Mexico City, Mexico
Tel.: +52-55/5606-4782
Fax: +52-55/5606-2282
E-Mail: jsotelo@servidor.unam.mx

Abstract The possible participation of different herpes viruses was studied during exacerbations of multiple sclerosis (MS). We searched for the presence of DNA from the following herpes viruses: varicella zoster virus (VZV), herpes-simplex viruses 1 and 2; Epstein-Barr virus (EBV) and human herpes-virus-6 (HHV6) in mononuclear cells from patients with MS during relapse (n = 40), MS during remission (n = 131) and controls (n = 125). Additionally, immune cells containing viral antigens were quantified by flow cytometry, and VZV load was determined by real time PCR in 2 MS patients at various times during relapse and remission. DNA from VZV was found in 95% of MS patients during relapse and in 17% during remission; all controls were negative; by contrast, DNA from HHV6 was found in 24% of MS patients during relapse and in 2% during remission; DNA from herpes simplex viruses was not found in any subject; and DNA from EBV was found in a similar percentage of subjects from all groups. Sequential quantification of VZV-load showed a curve that increased during relapse and disappeared at remission. Also, VZV antigens were found inside a large number of immune cells from MS patients during relapse as compared with MS patients on remission and controls. In the typical forms of VZV infection, varicella and herpes-zoster, DNA from VZV is found in mononuclear cells exclusively during brief periods at the beginning of the active infection, but not during latency; thus, the conspicuous presence of VZV during relapses of MS may indicate a period of active infection and suggests the participation of VZV in the pathogenesis of MS.

Key words varicella-zoster virus · multiple sclerosis · viral pathogenicity · herpes viruses

Introduction

A possible viral etiology for multiple sclerosis (MS) has long been suspected. In a preliminary study, we reported the transient appearance of DNA from varicella-zoster virus (VZV) in peripheral blood mononuclear cells (PBMC) from patients with MS during relapse [27]. In order to elucidate whether the VZV participates in the pathogenesis of MS we conducted a study which included a large cohort of MS patients and controls, the extensive search for 5 genes of VZV

Table 1 Base line characteristics of patients with MS according to disease activity

	Relapse (n = 40)	Remission (n = 131)	p
Age (years)	32 ± 2	33 ± 2	0.2
Gender M/F	10/30	49/82	0.1
Age at MS onset (years)	26 ± 2	28 ± 2	0.3
Years of evolution	6 ± 1	5 ± 0.5	0.5
No. relapses per year	2 ± 0.3	2 ± 0.1	0.2
EDSS	3 ± 0.4	3 ± 0.5	0.5

in PBMC, and flow cytometry of immune cells containing VZV antigens. Additionally, we investigated in the same patients the DNA from other herpes viruses: herpes simplex viruses (HSV) 1 and 2, Epstein-Barr virus (EBV) and human herpes virus 6 (HHV6), which have also been postulated as potential participants in the pathogenesis of MS.

Methods

Blood specimens from 131 patients with diagnosis of definite, relapsing-remitting MS were studied; 40 specimens were from patients within the first week of an acute relapse, while 131 were from patients on remission; of them, 91 were from patients tested only during remission and 40 were from the patients that had been studied during relapse who were tested again two months later, during remission. Clinical criteria for selection of patients within the first week of an acute relapse was; the occurrence, recurrence or worsening of symptoms of neurological dysfunction that lasted more than 24 hours and that stabilized or eventually resolved either partially or completely [27]. All MS patients included as "in remission" showed from 2 to 24 months of stability without clinical evidence of disease activity. No patient with progressive forms of MS was included, nor patients under interferon therapy. As controls, two groups were included, 60 healthy subjects and 70 patients with a comprehensive variety of neurological and immunological ailments (6 brain tumor, 6 stroke, 36 epilepsy, 10 migraine, 3 neurocysticercosis, 4 acute polyneuritis and 5 rheumatoid arthritis). Mean age of MS patients was 33 ± 8 years (15 to 60), 82 were females and 49 were males. No significant differences, besides disease activity, were found between the group of MS patients in relapse from those in remission (Table 1). Healthy controls had a mean age of 36 ± 8 years (18 to 56) male/female ratio was 46/14; control patients had a mean age of 37 ± 9 years (17 to 63) male/female ratio was 24/46. This investigation was approved by the Institutional review boards for research and ethics.

Search for viral DNA in PBMC was conducted by polymerase chain reaction (PCR) as described by Terada et al. [43] in PBMC separated by gradient centrifugation. The primers were designed using the DNA star software obtained from the Gene Bank of the National Center for Biotechnology Information (USA) for oligonucleotides from 5 genes of the VZV; the gene gD from HSV1 and HSV2; the genes LMP and gp85 from EBV; and the genes U29 and U67 from HHV6 (Table 2). To examine DNA integrity, the sequences ACACAACTGTGTTCACTAGC (nucleotides 180–199) and GGAAAATAGACCAATAGGCTG (nucleotides 430–410) from the β-globin gene, were included. All primers were synthesized by Bio-Synthesis (Mexico). The ORF's selected for this study have been proposed as specific and sensitive for detection of their respective herpes viruses, all nucleotide selected had no analogy with other herpes viruses as searched in BLAST (basic local alignment search tool), providing a high diagnostic precision. Viral isolates were used as positive control for each virus tested. Additionally, for VZV two positive controls were used, one from a varicella patient and one from a zoster patient they both were positive for all VZV primers tested.

After the results of single step PCR were obtained, serial quantification of viral load was made by real-time PCR as described by Ito et al. [17] in two of the last patients studied (cases 39 and 40, table 3) at various times starting during acute relapse until remission (Fig. 1). The primers and probe for the real-time relative quantification PCR assay were derived from the strain ORF31 from VZV and designed using Primer Express Software V 2.0 (Applied Biosystem), the forward primer and reverse primer were, (5′-CAC AAA AAC ACC CGA CTC GAA-3′) and (5′-ATT GGC ACG AAC TCA ACT G-3′) respectively, both were designed to amplify a target 65-bp fragment from the VZV. The TaqMan probe (1299T) was purchased from Applied Biosystem (Mexico). Each 25 μl PCR mixture contained 100ng of DNA from mononuclear cells in 5 μl of distilled water, 12.5 μl of TaqMan universal PCR Master mix (Applied Biosystem) and 1.25 μl of primer mixture. Human RNASA P was used as internal control. 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 60°C for 1 min, using an ABI PRISM 7500 real-time PCR system (Applied Biosystem). Amplified products were determined by continuous monitoring of fluorescence. After data collection, the cycle threshold (Ct) number was calculated by determining the point at which the fluorescence exceeded an arbitrary lower limit, chosen to cover the range of readings given by all standards in the exponential phase; the Ct value therefore reflected the overall quantity of target copies in samples. Each sample was run in triplicate and considered positive only if at least two of three results exceeded the threshold. For calculation of viral load the value of 1 was given to the results obtained on the first sample, against which the results obtained in subsequent samples were compared according to the following formula: $2\text{-}\Delta\Delta ct$ where ΔcT = media of cT from the gB gene - media from the constitutive gene (RNASA P); and $\Delta\Delta cT = \Delta cT$ of samples taken at different times - ΔcT of the initial sample.

The presence of VZV antigens within immune cells was measured by intracellular staining through flow cytometry as described by Snoeck et al. [34]: PBMC from blood specimens were obtained by gradient centrifugation with ficoll-histopaque 1077 (Sigma Chem. USA). Flow cytometry was made in samples from 17 MS patients during relapse, in 33 during remission and in 68 controls.

All samples to be tested by PCR and flow cytometry were codified before analysis and randomly included, so that the origin of each sample was not known at the time of the assay; the group source of each sample was decodified after the results were obtained. Statistical comparisons were made with the SPSS 10 software using the X^2 Test for categorical variables and the student's t test for continuous variables.

Results

Baseline characteristics of MS from patients studied during relapse or during remission were similar (Table 1); DNA from VZV was present in PBMC of most patients tested during relapse (95%) (Table 3), whereas it was found in 17% of MS patients tested during remission; all controls tested negative ($p < 0.0001$) (Table 4). The most frequently found VZV gene in MS during relapse was ORF68 (65%) followed by ORF4 (63%), ORF10 (53%), ORF31

Table 2 oligonucleotide primers

Target	Gene	Sequence (5′ to 3′)	Nucleotides
VZV	ORF4	GCGATTTTCCAAGAGAGACG	498–518
		TGTGGCATATCGGACTACCA	675–655
	ORF10	GCTACCGGTCACATGGAACT	1585–1605
		TTCACAGACCGCGATGTAAG	1800–1780
	ORF31	TACGTCCGTGAAATCGCAGTCCAT	1831–1854
		CCAGTCCCGCCAAAACCAATAATC	2233–2210
	ORF63	CGCACTGGAATGTGACGTAT	450–470
		TCCCCGTCTCGATAACAATC	653–633
	ORF68	AGATTGAACCGGGTGTCTTG	839–859
		CGCATTGGTTGACATGTAGG	1172–1152
HSV1	gD	CAGCAGGGGGTGACGGTGGACAG	700–722
		TGCGCTTTGGGGCTTTTTGAGTGC	1123–1100
HSV2	gD	CCCCGGGGTGAAGCGTGTT	195–213
		TTCGGGGATAAAGCGGGGTAGCAT	780–757
EBV	LMP	GGGCCCGCCTTTGATGACAG	1752–1771
		CCCGGGGCCTCGCTACCT	2153–2136
	gp85	GGCTCTTCTCACC	1418–1431
		TCACATTGATGAG	1651–1638
HHV6	U29	TTGTCTGTTGTCATGCGTCA	453–472
		TCCCATACTGGAGCTTTGCT	634–615
	U67	AAGCTTGCACAATGCCAAAAAACAG	17405–17429
		CTCGACTATGCCGAGACCCCTAATC	17627–17603

(50%), and ORF63 (40%) (Table 3). All nucleotides from the VZV genes ORF4, ORF31, and ORF63 were negative in MS patients who were on remission at the time of the study; in them, only the genes ORF10 or ORF68 were occasionally positive (8% and 9%, respectively). All patients with MS studied during relapse became negative for the 5 VZV genes when tested again during remission.

DNA from HHV6 was found in 9 (23%) MS patients on relapse and in 3 (2%) MS patients on remission but it was not found in controls ($p < 0.03$), positive samples for HHV6 DNA from MS patients at relapse corresponded to cases 4, 7, 11, 12, 17, 22, 23, 24 and 25 (Table 3), in all positive samples both genes, U29 and U67, were found (Table 4). Search for HSV1 and HSV2 was negative in all samples; whereas DNA from EBV was found in about half of all subjects studied, MS patients and controls, without significant differences between groups (Table 4).

Quantitative follow-up of DNA of the gene gB from VZV (ORF31) in two MS patients showed the presence of the viral DNA two days after the beginning of a relapse (at the first measurement) increasing during the first week and decreasing afterwards; two months later, during remission, the two patients tested negative (Fig. 1).

Flow cytometry (Fig. 2) showed a significant percentage of lymphocytes positive for VZV antigens in patients with MS during relapse (13.5 ± 3.3) as compared with MS during remission (5.4 ± 1.4 $p < 0.029$) or with controls (1.8 ± 0.5 $p < 0.0001$) (Table 5).

Discussion

Our findings support the idea that VZV participates in the etiopathogenesis of MS; VZV-DNA was found in 95% of MS patients during relapse. The presence of VZV was restricted almost entirely to patients who were within the first few days of relapse, the virus disappeared in all of them during remission. A quantification of viral load at various times in two MS patients illustrated this trend (Fig. 1). Few MS patients (17%) who were tested only during remission were also positive for a single VZV gene, while all controls were negative. Parallel to the findings by PCR, flow cytometry showed a significant number of lymphocytes containing VZV antigens at the time of clinical relapse of MS (Fig. 2); this reaction also decreased significantly during remission (Table 5). The absence of DNA and antigens from VZV in immune cells from most MS patients during remission, as well as in all controls, argues against the possibility of a casual association and suggests the participation of VZV in the pathogenesis of MS.

Similar to these findings, in the case of the typical infections caused by VZV (varicella and zoster) the viremia is transient and DNA from VZV is found in PBMC only during a brief period at the beginning of the disease, disappearing one week after the onset of rash. Also, serum antibodies in most cases do not increase sensibly during the acute infection [21]. Although VZV remains latent in nerve ganglia practically in all subjects after varicella infection, DNA from

Table 3 base line characteristics and pcr results of 40 patients with ms studied during relapse

Patient	Gender/ Age(years)	Evolution (years)	Varicella history /Age of infection	Herpes zoster history	Yearly mean of relapse episodes	EDDS	Current treatment	Relapse onset (days)	Cytometry*	VZV DNA				
										ORF 4	ORF 10	ORF 31	ORF 63	ORF 68
1	M/19	1.5	+/6	–	2	0	AZA	1	ND	–	–	+	+	–
2	F/30	8	+/2	–	4	8	AZA	2	ND	–	–	+	+	–
3	F/25	3	+/20	–	1	3	None	2	ND	+	+	+	+	–
4	M/25	2	+/9	–	1	2	MP	2	ND	–	–	+	+	–
5	M/34	8	–	+/34	3	2	MP	2	ND	+	–	–	–	+
6	M/21	3	+/5	–	6	5	None	3	ND	+	+	–	+	–
7	F/35	19	–	–	2	6	None	3	ND	–	+	–	+	–
8	F/44	9	+/2	–	1	3	MP	4	ND	+	–	+	+	+
9	F/44	26	+/3	–	2	2	AZA	4	ND	+	+	+	–	+
10	F/40	3	+/8	–	1	2	MP	5	ND	–	–	+	–	–
11	F/24	12	+/2	–	1	7	None	5	ND	–	–	–	+	–
12	F/40	10	+/8	–	4	7	None	5	ND	–	–	–	+	+
13	F/41	1	+/6	–	2	1	MP	6	ND	–	–	+	+	–
14	F/26	7	–	–	4	4	MP	7	ND	+	–	–	–	+
15	F/23	7	+/8	–	2	2	MP	7	ND	–	–	+	–	–
16	F/22	7	+/8	–	2	1.5	None	2	ND	+	–	–	+	+
17	F/36	1	–	–	2	1	None	7	ND	–	–	–	–	–
18	F/18	4	+/14	–	2	6.5	MP	2	ND	–	–	–	–	+
19	F/29	1	+/12	–	1	1	None	2	ND	–	–	–	+	+
20	F/25	1	+/5	–	2	2	AZ	4	ND	–	–	–	+	+
21	F/25	1	+/2	–	2	1	None	3	ND	–	–	–	–	–
22	F/27	1	+/18	–	1	1.5	None	1	6	–	–	–	+	+
23	M/44	10	–	–	1	3	None	7	27	+	+	–	–	+
24	M/33	6	–	–	2	5.5	None	3	7	+	+	–	–	+
25	F/24	8	+/7	–	1	1	None	1	35	+	+	+	–	+
26	F/43	2	+/5	–	1	2	AZ	3	41	+	+	–	–	+
27	F/44	3	+/5	–	2	1	AZ	5	6	+	+	–	–	+
28	F/15	1	+/8	–	2	1	None	2	2	+	+	–	–	+
29	F/44	11	+/2	–	3	7	None	3	0	+	+	+	–	–
30	M/46	1	+/5	–	2	2	None	7	28	+	+	+	–	+
31	M/21	1	+/10	–	2	1.5	None	3	6	+	+	+	–	+
32	F/43	10	+/4	–	1	2	None	5	11	+	+	+	–	+
33	F/26	11	+/6	–	3	8	None	6	1	+	+	+	–	+
34	F/19	1	–	–	2	1	MP	4	8	+	+	–	–	+
35	F/19	5	+/11	–	3	3	None	3	28	+	+	+	–	+
36	F/36	1	–	–	2	1	MP + AZ	3	0	+	+	+	–	+
37	F/30	5	–	–	1	2	AZ + MP	2	1	+	+	–	+	+
38	F/36	1.5	–	–	2	5	None	7	0	+	–	+	+	–
39	M/22	2	+/7	–	2	3	None	3	12	+	+	+	–	+
40	M/59	10	+/5	+/59	4	4	None	2	14	+	+	+	–	+

EDSS = extended disability status scale; AZA = azathioprine; MP = methylprednisolone; ND = not done; – = negative.
* Flow cytometry was made to 18 patients, results are expressed as the percentage of lymphocytes positive for VZV antigens

VZV is not found in PBMC from individuals without productive infection [21]. Even in cases of acute VZV infection of the brain virus recovery from PBMC is so uncommon after the initial days that the diagnosis relies mostly on the demonstration of intrathecal synthesis of oligoclonal antibodies [12]. In cases of immunization with high doses of attenuated VZV the virus can be recovered from PBMC only during the first 4–7 days, disappearing afterwards [39]. Occasionally, acute VZV cerebral infection develops months after the occurrence of zoster [11, 12], suggesting that VZV may also remain latent in central neurons, as it does in sensory neurons. Also, latent VZV has the capacity to activate and spread beyond ganglia to the spinal cord and brain [8, 11, 36].

By contrast with the conspicuous presence of VZV in most MS patients during relapse the HHV6 was found in one from every five MS patients, a figure similar to others reported [2, 35, 42]. During latency, HHV6 is mostly lymphotropic [31] while VZV is mostly neurotrophic; therefore, we believe that our findings suggest a closer association of VZV with MS. Other herpes viruses, frequent in humans in latent form, such as HSV1 and 2 and EBV [46] did not show similar participation in exacerbations of MS, providing additional support to the idea that the singular

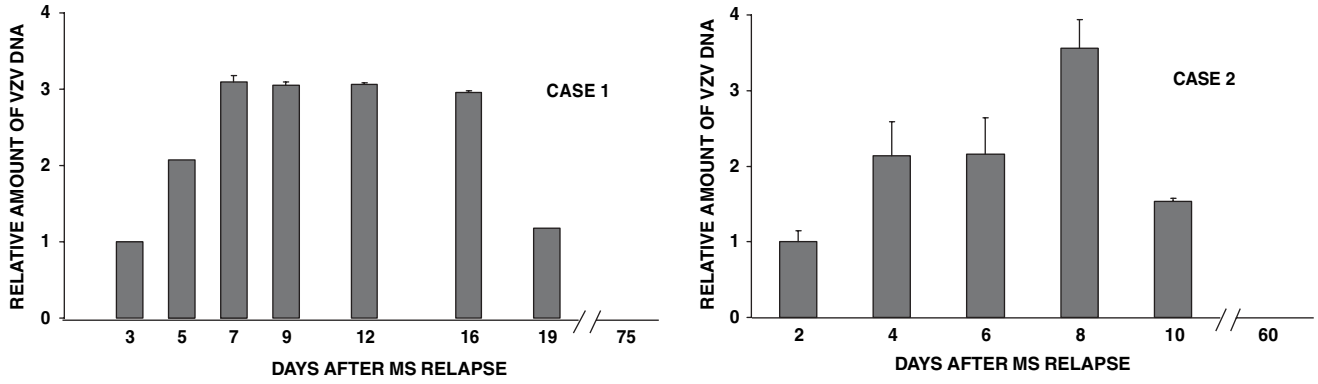

Fig. 1 Real-time relative quantification of viral load by PCR assay of gene ORF31 (gene gβ) from VZV in two MS patients (cases 39 and 40, table 3) at different times starting on days 3 (left) and 2 (right) after the beginning of clinical relapse, the final sample was taken on days 60 and 75, respectively. In both cases, the viral load doubled by day 4 and tripled by day 8 as compared with the initial measurement (Ct value), decreasing afterwards; two months later, viral DNA could not be detected

presence of VZV is not an epiphenomenon of simultaneous viral activation due to the immune disturbances and immunosuppressive treatment that accompany episodes of MS exacerbation. Additionally, DNA from VZV was absent in all control patients, some of them had inflammatory or immune-mediated disorders (neurocysticercosis, acute polyneuritis, rheumatoid arthritis) or were exposed to immunosuppressant therapy (brain tumor treated with chemo- and radio-therapy as well as steroids) these results argue against the possibility of a non etiologically-related epiphenomenon of viral reactivation of VZV in MS patients [27]. However, the association of VZV positivity and MS relapses might also be the result of another factor, e.g. infection with another agent which causes MS exacerbation and VZV activation, or that the immune activation during an MS relapse might lead to VZV reactivation.

Table 4 pcr results for dna from genes of varicella zoster virus, epstein barr virus, herpes simplex viruses and human herpes-virus 6 in mononuclear cells from patients with ms and controls

VIRUS/GENE	MS IN RELAPSE n = 40 (%)	MS IN REMISSION n = 131 (%)	CONTROLS n = 130 (%)
VZV			
ORF4	25 (63)	0	0
ORF10	21 (53)	10 (8)	0
ORF31	20 (50)	0	0
ORF63	16 (40)	0	0
ORF68	26 (65)	12 (9)	0
POSITIVE FOR ANY GENE	38 (95)	22 (17)	0
EBV			
gp85	17 (43)	61 (47)	23 (18)
LMP	12 (30)	54 (41)	59 (46)
POSITIVE FOR ANY GENE	19 (48)	79 (60)	67 (52)
HHV6			
U29	9 (23)	3 (2)	0
U67	9 (23)	3 (2)	0
POSITIVE FOR ANY GENE	9 (23)	3 (2)	0
HSVs			
1 gD	0	0	0
2 gD	0	0	0

Countless reports have speculated for the last 40 years on a possible viral etiology for MS [37]. However, most initial evidence has been negative in confirmatory studies. The peculiar ability of some herpes viruses to produce in humans recurrent exacerbations and remain latent for long periods [7] have provided a fair theoretical framework for research since the 1960s [19]; along the years, many herpes viruses have been postulated, particularly EBV, HSV1, 2, HHV6 and VZV [2, 30, 38, 47]; however, most laboratory or subsequent studies have been either inconclusive or negative [19, 23, 24, 37]. In the case of VZV some epidemiological inferences have been made [30, 41], however, the ubiquitous presence of the virus in humans, either in the latent stage within neural cells from sensory ganglia or in common diseases such as varicella, which is almost universal in countries endemic for MS, provide confusing evidence and the associations remain controversial. Comprehensive reviews on the subject have concluded that confirming or refuting the possible participation of VZV in the etiopathogenesis of MS is difficult and could not be supported on the light of previous studies [10, 22, 25, 33].

Failure to identify VZV by previous studies, either by virus isolation in culture, by disease transmission in experimental animals or by immunodiagnosis might be due to the peculiar characteristics of VZV: a) VZV has a strong cell-associated nature, which makes it extremely difficult to isolate the virus in culture [7, 8, 31, 36, 37, 46]; b) it has a peculiar species-specificity for humans that prevents its transmission to experimental animals; c) antibodies to VZV are present in

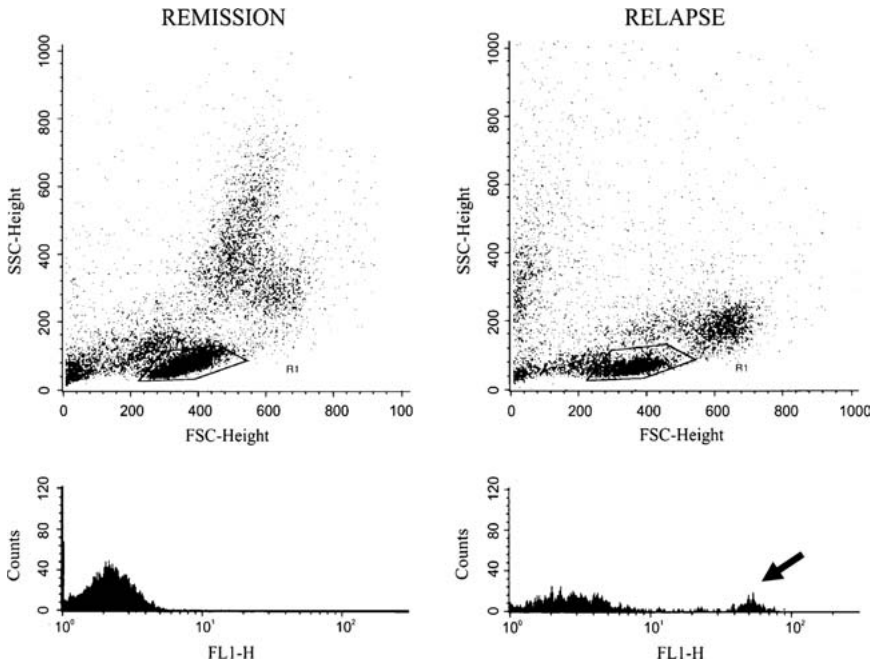

Fig. 2 Flow cytometry analysis of expression of intracellular VZV antigens on PBMC from a patient with MS (case 26, table 3) during remission (left) and relapse (right); cells were stained with fluorescent antibodies against VZV proteins. Histograms are from a representative MS case during relapse; the arrow shows a high fluorescent intensity of lymphocytes marked with antibodies to VZV

Table 5 flow cytometry for varicella-zoster virus in mononuclear cells from patients with ms and controls

GROUP	MEAN (±SE)	P VALUE
A) MS in relapse (n = 19)	13.5 ± 3.3	A/B 0.025 A/C 0.000
B) MS in remission (n = 33)	5.4 ± 1.4	B/A 0.025 B/C 0.005
C) Controls (n = 68)	1.8 ± 0.5	C/A 0.000 C/B 0.005

the serum from most healthy subjects, these antibodies do not increase sensibly during episodes of VZV infection, making immunodiagnostic tests in serum of limited value in all acute VZV infections [7, 8, 11, 44]; finally, d) the period of viremia in the typical VZV infections, varicella and zoster, is brief and restricted to few days at the beginning of infection [3, 7, 12, 21, 29]. Also, in contrast with other studies that have searched the virus by PCR technology, we separated the MS cases in two subgroups, relapse and remission, under the assumption that the causal factor for MS could be particularly evident during exacerbations and might either diminish or disappear during remissions, our results showed that in fact, the presence of the virus is critically limited to the initial days of exacerbation but not during the long periods of remission.

Mexico is geographically located in tropical and subtropical regions; coincidentally, a north-south diminishing gradient becomes evident in this country for both diseases, MS and varicella [1, 20], with lower incidence than in countries located in the northern-hemisphere with temperate climate, where varicella is almost universal at early ages and MS is endemic [6, 18, 26]. Nonetheless, a recent trend of higher than usual incidence in Mexico for both diseases, MS and varicella, has been documented [13, 41]. In Mexican patients with MS the antecedent of varicella infection during childhood and adolescence constitutes the main risk factor for MS as compared with paired controls and with the general population, in whom the history of varicella infection is found in less than fifty percent [41].

From the 5 DNA segments from VZV studied, most positive patients had various, but not all, the absence of other DNA segments in the same patient can be explained by the fact that immune cells traffic through tissues with active infection and engulf viruses whose DNA might then be amplified [3, 11]. Thus, our results suggest that, as with zoster infection, VZV is not in active replication in PBMC but in the process of degradation after phagocytosis from a distant active infection.

Recent reports showing early axonal pathology in MS, not secondary to demyelination, which seems to be relentless and cumulative even in areas of normal white matter [26]. This supports the hypothesis of viral infection of central neurons, in which VZV produces early axonopathy followed by demyelination; this combination is a peculiar feature of acute CNS infections caused by VZV [5, 11, 12]. Interferon therapy is effective in the primary VZV infections,

chickenpox and zoster; interestingly, it is also effective in MS, although the mechanisms for this have not been clarified [4, 10, 11, 16, 29]. Some patients develop MS soon after varicella or zoster infection [28, 29]. The VZV has a distinctive ability for multiple pathogenicity related to the age of the host and to the cells infected [36]; in this way, the same virus may cause either chickenpox or herpes zoster or, we speculate, MS.

On the basis of our findings, we construct the following hypothesis: VZV is acquired early in life and remains latent in neural cells; in a susceptible individual the virus is activated and spreads during MS relapses from the neural ganglia to central neurons, where viral proteins are expressed, provoking a local immune reaction that induces demyelination and axonal pathology; through this reaction PBMC phagocytize the viruses and eliminate the active infection but leave immune-mediated tissue damage that is partly repaired, with remaining neurological sequelae. Afterwards, the VZV returns to latency during remission. To elaborate further on this hypothesis complex challenges exist: *i*) viral replication at the site of the plaques should be demonstrated; *ii*) due to the species-specificity of VZV, restricted to humans, the development of experimental models to reproduce an animal form of MS by VZV infection would be difficult; *iii*) attempts to prove VZV participation by therapeutic trials with antivirals [4, 45] are also difficult, as the drugs are ineffective during viral latency and the therapeutic window would be restricted only to the initial days of MS exacerbation; *iiii*) the only available prophylactic vaccine uses a live attenuated VZV (Oka strain) which can also remain latent within the nervous system for long periods and, theoretically, could also induce MS in susceptible individuals, as has been demonstrated for varicella and herpes zoster [9, 32, 40]; the use of an inactivated varicella vaccine could overcome this circumstance [14, 15]. Currently, we are conducting similar studies in cerebrospinal fluid and in patients with progressive forms of MS.

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