Brief Presence of Varicella-zoster Viral DNA in Mononuclear Cells During Relapses of Multiple Sclerosis

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Background: A possible viral cause for multiple sclerosis (MS) has long been suspected. A progressive increase in MS has been reported in Mexico during the past 20 years; a conspicuous antecedent of varicella infection during childhood has been the most relevant finding in the medical history of patients with MS.

Objective: To investigate the possible participation of varicella-zoster virus (VZV) in the etiopathogenesis of MS.

Design, Setting, and Patients: We searched, by polymerase chain reaction (PCR), for VZV DNA in peripheral mononuclear cells of 82 patients with relapsing-remitting MS. Additionally, genes gD from herpes simplex viruses 1 and 2 were sought by PCR, as well as IgG and IgM serum antibodies to VZV.

Results: Viral DNA from the genes open reading frame (ORF)31, ORF62, ORF63, and ORF67 of VZV was found in mononuclear cells from 13 (87%) of 15 patients with MS who were tested during acute relapse. All patients who were tested during remission (n = 67) were negative for the DNA, including patients who were initially positive and were tested again after 2 months of remission. All control patients with a comprehensive variety of neurologic diseases (n = 100) and healthy controls (n = 20) also tested negative. All subjects were negative for herpes simplex viruses 1 and 2 DNA, and no differences were found in serum antibodies to VZV.

Conclusions: The finding of genes of VZV in peripheral mononuclear cells, restricted to a brief period during clinical relapse of MS, suggests either its participation in the etiopathogenesis of MS or an epiphenomenon of viral activation simultaneous with the relapse of MS.

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Methods

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Many viruses have been associated with multiple sclerosis (MS). Among them are those from the family α-herpesvirus. In Mexico, a sharp and progressive increase in the incidence of MS during the past 20 years has been reported, making this country a good location for research on etiologic factors. We have found varicella infection as the most significant risk factor in the medical history of patients with MS. Thus, the present study aimed to detect the presence of varicella-zoster virus (VZV) in patients with MS.

We studied DNA from various genes of VZV in mononuclear cells from 82 consecutive patients with a diagnosis of relapsing-remitting MS; 33 were male and 49 were female, with age ranging from 16 to 57 years (mean ± SD, 31 ± 9 years). All fulfilled the diagnostic criteria for clinically definite MS; 15 of them (18%) were included in the study within the first week of an acute relapse, and the rest were in remission. Relapses of MS were defined as reported by Confavreux et al: “the occurrence, the recurrence or the worsening of symptoms of neurologic dysfunction that lasted more than 24 hours and that stabilized or eventually resolved either partially or completely.” Patients with a diagnosis of progressive MS were not included. Twenty healthy subjects and 100 patients with a comprehensive variety of neurologic disorders, which included neurocysticercosis (12 patients), neoplasm (8), migraine (32), Guillain-Barré syndrome (4), amyotrophic lateral sclerosis (2), and epilepsy (42), were included as controls; 52 were male and 68 were female, with ages ranging from 18 to 56 years (mean ± SD, 37 ± 12 years).

Search for VZV DNA in mononuclear cells was conducted by polymerase chain reaction (PCR) in a blinded fashion in codified samples. Five milliliters of peripheral blood was treated with 0.02 mL of EDTA. Mononuclear cells were separated by gradient centrifugation with Ficoll, and DNA was extracted with a mixture of...
Phenol, chloroform, and isoamyl alcohol and precipitated with isopropanol. The DNA was dried at room temperature and dissolved in 100 µL of sterile water. The PCR amplification was performed with 50 µL of a mixture of 0.5 mM magnesium chloride, 200 µM deoxyribonucleoside triphosphates, 0.2 µM primers, 1 U of Taq polymerase, and 0.6 µg of DNA (PerkinElmer, Boston, Mass). It was covered with 50 µL of mineral oil. The primers were designed with the DNA Star software (Sowden J. National Institutes of Medical Research, London, England) for the sequences of ORF 63; and the sequences TCCACGTTACCCGAAAAGTC (nucleotides 775-794) and GGATTCTGGTGTCGCATTTT (nucleotides 975-956) of the gene open reading frame (ORF67); the sequences CACGTGGAGAAAAACCGAAAACCATATTGC (nucleotides 1021-1040) and ACCACCACTACATTGTAGCC (nucleotides 1219-1021) of the gene ORF62; and the sequences CGCAGGTGAAGACTGCTAT (nucleotides 450-470) and TCCCCGTCCTCGATAAATC (nucleotides 653-633) of the gene ORF63. To examine DNA integrity, the β-globin gene was included: ACAAATCGTGGTCTAGC (nucleotides 180-199) and GGAAATAGACCAATAGGCTG (nucleotides 430-410), obtained from the GenBank of the National Center for Biotechnology Information (National Library of Medicine, Bethesda, Md). All primers were synthesized by Bio-Synthesis (Mexico City, Mexico). Thirty amplification cycles were made in a thermocycler (Eppendorf master cycler 5330; Eppendorf AG, Hamburg, Germany) as follows: 94°C (denaturation), 55°C (annealing), and 72°C (extension) during 45, 45, and 90 seconds, respectively, with a final extension of 1 cycle of 7 minutes at 72°C for VZV; annealing for ORF 62, ORF 63, and ORF 67 was carried out at 60°C. For the β-globin gene, 23 cycles were made under the foregoing conditions except for 58°C for annealing. Amplified PCR products were visualized in 2% agarose gels stained with ethidium bromide and run for 1 hour at 100 V in Tris-borate-EDTA buffer 1X. To monitor contamination, a water control was included.

After the results of VZV DNA were obtained, we used identical methods to search the genes gB from herpes simplex viruses 1 and 2, with the following sequences: CCCCCGGGT-GAAAGCTT (nucleotides 195-213) and TTCCGGG-GATAAGCCGGGTGATCAT (nucleotides 757-780) and the sequences CACCGAGGGTGGACCGGTTGACAG (nucleotides 700-722) and TGCCTTTGGGCTTCTTGAGTGC (nucleotides 1100-1123).

Serum from patients with MS and 65 healthy control subjects was searched for IgG and IgM antibodies to VZV antigens by enzyme-linked immunosorbent assay (DiaSorin, Kasel, Germany). Serum diluted 1:200 was reacted for 60 minutes at room temperature with VZV antigens coupled in the plate wells and washed 3 times with phosphate-buffered saline–Tween 20, then 100 µL of either anti-IgG-HRP or anti-IgM-horseradish peroxidase was added and washed. After 30 minutes, the substrate reaction was stopped with tetramethylbenzidine for 15 minutes, stopped with 1M sulfuric acid, and read at 450 nm. Positive samples were considered those with an optical density greater than 0.6. Mean optical density values obtained from patients with MS during remission, from patients with MS during exacerbation, and from controls were compared by analysis of variance.

RESULTS

Viral DNA from VZV was found in mononuclear cells from 13 (87%) of 15 patients with MS who were within the first week of an acute relapse at the time of the study (Figure). The gene gB ORF 31 was found in 9 patients, ORF 63 was found in 10, ORF 62 was found in 2, and ORF 67 was found in 1 (Table). New samples taken again from these positive cases when the disease was in remission tested negative. All samples from patients with MS who entered the study during remission (n = 67) were negative for all VZV genes studied; in these patients, the time elapsed after the last clinical relapse of MS varied from 2 to 48 months. All samples from controls (n = 120) were also negative. All PCR tests were repeated on 3 occasions, with identical results.

Patients with MS who tested positive for VZV DNA in mononuclear cells had entered the study a mean of 3.9 days (range, 1-7 days) after the beginning of clinical signs of an acute relapse (Table). None of them was receiving interferon therapy. Other clinical and demographic features were indistinguishable from those of patients with MS who entered the study during periods of remission.

Serum testing for IgG antibodies to VZV was positive in 63 (77%) of patients with MS and in 94 (78%) of controls (P = .17); mean optical density values of the enzyme-linked immunosorbent assay were higher in patients with MS than in controls, but without statistical significance (1.36±0.7 vs 1.05±0.6). Mean VZV antibody titers in serum were also higher in patients with MS positive for VZV DNA than in the rest of the patients with MS, but again, without significance (1.36±0.7 vs 1.05±0.6). Likewise, no significant differences in antibody titers were found in patients with MS positive for VZV DNA than in the rest of the patients with MS, but again, without significance (1.36±0.7 vs 1.05±0.6).
MS during remission were 0.34 ± 0.02 and 0.36 ± 0.04 during relapse (not significant).

**COMMENT**

Our findings suggest that either latent VZV is activated during an MS relapse, as a simultaneous epiphenomenon, or VZV participates in the etiopathogenesis of MS. It is apparent that the presence of viral DNA in mononuclear cells is short lived and restricted only to the initial days of disease relapse. The absence of herpes simplex viral DNA argues against the possibility of associated virus reactivation not involved in the etiology. If VZV is directly related to MS, it could be speculated that in some patients with MS, VZV may be latent within the central nervous system and periodically reactivated, inducing either immunologically enhanced cell damage or a short-lived phenomenon of molecular mimicry, triggering an autoimmune response against myelin proteins. Within this possibility, the participation of VZV in MS would represent an interesting trail in the intricate mechanisms of multiple viral pathogenicity in which the same virus might cause, at different times and under different circumstances, either a systemic primary infection (varicella) or 2 different forms of recurrent local infection within the nervous system (herpes zoster and MS), each depending on the cell group that harbors the latent infection.

The VZV has a high degree of genetic stability with little antigenic diversity among isolates; the ORF63 gene encodes a glycoprotein of 45 kDa present in the tegument, whereas ORF62 encodes a virion-associated transactivator. The identification of VZV gene 63–encoded protein is highly specific and sensitive for the diagnosis of varicella and herpes zoster and is directly related to the amount of VZV DNA; this protein is a component of the virion and is expressed very early in the infection. Immediate early 63 protein is an important target of immunity to VZV; T-cell recognition of immediate early 63 is likely to be involved in controlling VZV reactivation from latency. Cotransfection experiments have demonstrated that VZV gene 63 protein represses the expression of VZV gene 62 and plays a pivotal role in the activation of early gene expression. The other viral DNA from gene ORF31 found in most of our patients with MS during relapse encodes the glycoprotein gB, abundant in VZV and expressed during infection, where it plays an important role for the entry of the virus into the cell; gB is a major viral antigen that elicits immunity and neutralizes antibodies. Amplification of these 2 viral genomic segments allows VZV detection with a high sensitivity and specificity.

According to our results, the presence of VZV DNA only during the initial days of clinical exacerbation could be similar to the characteristic diseases caused by VZV, varicella and herpes zoster, where the viral DNA can be detected in peripheral mononuclear cells only during the initial days of the acute stage. In varicella, the virus is rarely isolated; infected circulating cells are usually cleared within 24 to 72 hours after the appearance of rash. Additional features that may have masked the finding of VZV in MS in other studies despite a long-held clinical suspicion are the similar levels of serum antibodies against VZV in controls and patients with MS during either relapse or remission of the disease, found in our patients and in other similar studies; however, this circumstance is also conspicuous in the natural course of varicella and zoster infections. Also, the VZV is difficult to detect by culture of infected tissues because of its strong cell-associated nature with no virus release into supernatant. The host range of VZV is highly restricted to humans, which would explain the failure of several experimental attempts at disease transmission by inoculation of brain tissue from patients with MS into nonhuman hosts.

Similar to the epidemiology of MS, the epidemiology of varicella shows large differences between temperate and tropical areas and a north-to-south diminishing gradient, yet to be explained. In temperate regions, varicella during childhood is almost universal, whereas in subtropical and tropical regions, where Mexico is geographically located, varicella occurs at an older age and affects less than 50% of the general population. Nonetheless, coincidental with the continuous increase of MS in Mexico during the past 20 years, the incidence of varicella has decreased significantly. The VZV DNA amplification results of 15 patients during relapse (Table 1) show a higher percentage of DNA in varicella cases, which could be directly related to a higher incidence of VZV activation in MS during relapse.
cella has also had a sharp increase; the annual rate of infection per 100,000 population reported by the Ministry of Health in 1984 was 74, in 1990 it was 178, and by 2000 it had risen to 377; the rate quintupled within 15 years.

A peculiar property of VZV is that, after the primary infection, it establishes latent or asymptomatic infections in ganglia along the entire nervous system. This persists for the lifetime of the host; periodic episodes of subclinical reactivation probably occur, but latently infected cells escape destruction by the immune system. Most studies indicate that neurons are the primary, if not the exclusive, site of latent VZV; on reactivation, the virus may spread transaxonally. However, human astrocytes in culture have also been infected. Although VZV was isolated 30 years ago, varicella remains uncontrolled mainly because it is widely considered benign. The major complications of varicella infection occur in adult patients in whom VZV shows a clear tendency to become neurotrophic. Cells infected with VZV may lose virus-specific membrane antigens, avoiding immunologic recognition, which favors a latent infection with absence of circulating virus during long periods of containment. This could be the case of VZV in patients with MS during remission, when the virus might remain sequestered within brain cells in a latent state for long periods. Indirect support for the potential role of VZV in the etiology of MS is the therapeutic benefit obtained with interferons alfa and beta in the treatment of multiple sclerosis.20 The above-described pathogenic characteristics of VZV could help to explain the following peculiarities of MS: the clinical picture of relapse–remission, the absence of demonstrable virus during the long periods of containment, and the brief periods of clinical neurologic damage, apparently restricted to viral reactivation and immune response. Limitation of this study is the differential detection of genomic segments. In only 1 patient (case 8) we found all segments studied. The absence of 1 or more of these segments in the other positive cases could be due to differential degradation of viral DNA; it also seems possible that the presence of viral DNA within mononuclear cells was due to phagocytosis of VZV particles from a distant infection, rather than active viral replication in these cells.

We are currently studying RNA and gene products from VZV in a large group of patients with MS. Various questions must be investigated; to discern a causative role of VZV in MS, the presence of VZV must be studied in patients from other latitudes and different ethnic backgrounds, particularly in areas of high prevalence of MS as well as in patients with the progressive forms of MS. If these initial results are replicated in similar studies, therapeutic trials with antiviral drugs or prevention through VZV immunization would be warranted; in both instances, previous experiences have shown encouraging results. However, VZV from the live attenuated vaccine currently in use might become latent and can reactivate, which theoretically would pose risks for MS development in susceptible individuals; also, the effectiveness of antiviral drugs on latent VZV infection is not known.

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