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Is Varicella Zoster Virus Really Involved in the Pathogenesis of Multiple Sclerosis?

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS). The cause of MS is unknown. Leading theories are that MS is infectious or has a virus-triggered immunopathology, possibly with an autoimmune component. Current pathological studies suggest that apoptosis in oligodendrocytes may be an early event. Virus might reactivate after years of latency and kill oligodendrocytes, as occurs in progressive multifocal leukoencephalopathy, the only human demyelinating disease with a proven viral cause. The putative MS virus might also initiate an immunopathology leading to demyelination, as occurs in animals infected with certain strains of Theiler's murine encephalomyelitis virus, mouse hepatitis virus, or Visna virus.[1]

Numerous epidemiological studies provide presumptive evidence that MS is acquired.[2] Furthermore, studies of identical twins with one MS-afflicted individual show that only 30% of second twins experience development of disease, suggesting that more than a putative susceptible genotype determines disease.[3] However, the best evidence to support infection as the cause of MS is the increased amounts of IgG, manifest as oligoclonal bands, in the brain and cerebrospinal fluid (CSF) of more than 90% of patients with MS. The other human CNS diseases that display high concentrations of IgG and oligoclonal bands in brain and CSF are infectious,[4] and the oligoclonal IgG in those diseases have been shown to represent antibody directed against the causative agent. Indeed, the now classical study showing that the oligoclonal IgG in subacute sclerosing panencephalitis (SSPE) brain and CSF is directed against measles virus[5] was followed by demonstrations that the oligoclonal IgG in cryptococcal meningitis is directed against cryptococcus,[6] and that the oligoclonal IgG in neurosyphilis is directed against *Treponema pallidum*.[7] Thus, the oligoclonal IgG in MS brain and CSF is also likely to be directed against the agent/antigen that triggers MS.

From 1946 to 1994, there were multiple claims of recovery of virus, including rabies virus, various parainfluenza viruses, coronaviruses, retroviruses, and multiple human herpesviruses, from various MS tissues.[8] In the 1970s, Hilary Koprowski, Zofia Wroblewska, and I searched extensively for virus in MS brain. We removed plaque-periplaque areas where IgG is located in MS brain and propagated MS brain cells from those areas in tissue culture.[9] Using the same techniques that had successfully rescued herpes simplex virus (HSV) from human trigeminal, nodose, and vagus ganglia, we cocultivated MS brain cells with indicator cells (lung fibroblasts and kidney cells in which a virus might be more likely to replicate) in tissue culture; however, no cytopathic effect characteristic of any infectious agent developed. Because cell fusion provides more intimate contact between MS brain cells and indicator cells than cocultivation, we also used agents such as lysolecithin and polyethylene glycol to fuse the membranes of the MS brain cells with those of the indicator cells to produce a heterokaryon (a cell containing different nuclei inside a common cytoplasm), but the fused cells did not develop any spontaneous cytopathic effect. Indirect immunofluorescence analysis demonstrated no binding of either explanted or fused brain cells to antibody directed against measles virus, HSV, varicella zoster virus (VZV), cytomegalovirus, Coxsackie and ECHO viruses, rubella, influenza A and B, or coronaviruses. Our efforts to produce neurological disease by inoculating explanted and fused MS brain cells into embryonated

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hens' eggs, which readily support the growth of influenza and parainfluenza viruses, as well as into rodents and chimpanzees, also demonstrated no virus. In another strategy, we searched for an enveloped virus that might be latent in MS brain, based on Weiss and colleagues'[10] demonstration that superinfection with vesicular stomatitis virus (VSV) in tissue culture cells already infected with an enveloped virus creates a small fraction of virus containing the genome of one virus but the protein of the other virus and that is able to resist neutralization by antibody to VSV; however, inoculation of MS brain cells with VSV and subsequent analysis of the harvested cells for a VSV nonneutralizable fraction identified no virus. Overall, analysis of 24 brains by techniques available in the 1970s failed to identify virus or viral antigen in MS brain.[11] Nonetheless, it is important to recognize that MS appears to be an exclusively human disease, and that if a virus does cause MS, it may be an exclusively human virus that does not kill cells in tissue culture or produce disease in experimentally infected animals. Epstein–Barr virus is such an example.

Notwithstanding Willie Sutton's reason for robbing banks, that is, "because that's where the money is," the repeated unsuccessful attempts to detect virus in MS plaque-periplaque areas should perhaps have suggested the potential value of looking for virus in MS CSF. In this issue of *Annals of Neurology,* Sotelo and colleagues[12] report such studies on CSF and blood mononuclear cells (MNCs) of MS patients during relapse and in remission. Ultrastructural analysis of CSF obtained from 15 MS patients demonstrated the presence of virions the size and configuration of herpesvirus particles in CSF removed within 8 days after the onset of an exacerbation in all 15 patients. The hexagonal shape, reflecting the icosahedral symmetry and the capsid structure, appears to be highly representative of herpesvirus type particles. Ultrastructural examination of CSF obtained from MS patients during relapse and immunoprecipitation with antibody against three VZV glycoproteins also demonstrated herpesvirus virions. Because the eight known human herpesviruses cannot be distinguished morphologically, the investigators used polymerase chain reaction (PCR) with primers from five of the human herpesviruses (HSV-1, HSV-2, EBV, VZV, and human herpesvirus-6 [HHV-6]) to determine the herpesvirus present. PCR identified the herpesvirus as VZV and not any of the other herpesviruses studied. During exacerbation, real-time PCR showed that the viral DNA copy number was high in all 15 MS patients (mean of 204,489 [±26,782] copies/ml CSF). After relapse, the mean viral load in CSF decreased to 377 (±124) copies/ml, although minimal traces of VZV DNA were still detectable in most patients. CSF from 40 control patients, including some patients with inflammatory CNS disease, mostly cysticercosis, were negative for herpesvirus particles by electron microscopy (EM), and the VZV copy number in CSF from 25 of 28 control patients was ±3 copies/ml. Overall, these findings are remarkable, and if confirmed, strongly incriminate VZV in MS.

Because CSF is turned over several times a day, the presence of VZ virions in MS CSF indicates active infection. Although most cases of MS are characterized by remissions and exacerbations, MS is a chronic disease and oligoclonal IgG bands persist throughout the lifetime of the patient. Thus, it is surprising that Sotelo and colleagues[12] did not find differences in anti-VZV IgG and IgM antibodies in CSF of MS patients compared with control patients. If VZV causes MS, then the increased amount of IgG in MS CSF (and brain) detected as oligoclonal IgG bands would likely be anti-VZV IgG antibody. Why did the enzyme-linked immunosorbent assay test that Sotelo and colleagues[12] used not show increased anti-VZV IgG in the MS patients, especially because their flow cytometric studies demonstrated a high percentage of blood MNCs that contain VZV antigens during an MS exacerbation?

Involvement of VZV in the pathogenesis of an MS plaque would help to explain the considerable abundance of cell-free VZV (the virions and high copy numbers of VZV DNA) in CSF, as well as the presence of VZV (most likely cell associated) in blood MNCs found by Sotelo and colleagues.[12] It also appears reasonable that the abundance of cell-free VZV in CSF found during an MS exacerbation would likely reflect the presence of VZV in plaques. Whereas EM studies of MS tissue at autopsy by multiple investigators have failed to detect herpesvirus particles,[13] MS plaques at autopsy might contain less free virus than would be present during MS exacerbation itself. Nevertheless, if VZV did generate an MS plaque to the extent that such an abundance of cell-free VZV is present in CSF, analysis of an MS plaque after death would likely demonstrate some footprints of an earlier VZV infection. Although our earlier prolonged search for viral antigen, including VZV, in MS plaques was negative,[11] if Sotelo and colleagues are correct, there should be amplifiable VZV DNA in active MS plaques to support their findings. Furthermore, although VZV is only rarely isolated from CSF of patients with neurological disease caused by VZV (eg, myelitis and vasculopathy), it appears possible that VZV might have occasionally been isolated from MS CSF during an exacerbation, given that such an abundance of cell-free virions can be seen by EM, the VZV DNA copy number in CSF is so high, and viral antigen is readily detected in blood MNCs. To my knowledge, VZV has not been isolated from MS CSF.

Could the presence of VZV in MS CSF be indicative of virus that reactivated nonspecifically from ganglia during the acute inflammatory process of an MS

exacerbation? Unlike HHV-6 and EBV, which become latent in T cells and B lymphocytes, respectively, VZV does not become latent in blood MNCs. Thus, the detection of VZV DNA in blood MNCs likely reflects trafficking of MNCs through tissue infected with VZV where they encounter and engulf virus whose DNA is then amplified by PCR. If VZV reactivated nonspecifically from ganglia and was engulfed by circulating blood MNCs, there might be some cell-associated virus present in MNCs. Even so, it is unlikely that such a large abundance of cell-free VZV would be found in CSF. Thus, future control patients must include ultrastructural examination of CSF from patients with non-MS inflammatory diseases and with even the modest CSF pleocytosis (10–20 cells) often encountered in MS. All but one of Sotelo and colleagues' non-MS inflammatory control patients were patients with cysticercosis, most of whom were presumably asymptomatic and unlikely to have had a CSF pleocytosis.

MS researchers should perform direct ultrastructural examination and PCR on CSF obtained from MS patients during exacerbation. The same EM and PCR analyses should be performed on CSF from patients with non-MS CNS inflammatory disease who have a CSF pleocytosis. Additional studies should try to absorb out the oligoclonal IgG in MS CSF by incubation with VZV, as Burgoon and colleagues[14] did in patients with CNS vasculopathy caused by VZV. Furthermore, MS brain plaques and periplaque areas can be examined readily by PCR for amplifiable VZV DNA. Finally, single-cell reverse transcriptase PCR repertoire analysis demonstrates that clonal expansion is a prominent feature of the B-cell response in MS CSF.[15] Recombinant antibodies have been produced from overrepresented heavy- and light-chain immunoglobulin sequences in patients with chronic infectious and inflammatory diseases of the nervous system, including MS and SSPE. In SSPE, such antibodies produced from brain[16] and CSF[17] are directed against measles virus, the cause of SSPE. The recombinant antibodies in MS should be examined quickly to determine whether they are directed against VZV. All of these studies can and should be done quickly to confirm or repudiate Sotelo and colleagues'[12] tantalizing findings.

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