EDITORIAL

Viral Pathogens in Multiple Sclerosis

An Intriguing (Hi)story

PHYSICIANS AND PATIENTS HAVE ALWAYS HAD A great fascination with the invisible pathogen. Koch's criteria,[1] set out as early as 1882, have generally been ignored, that is, a microorganism must be (1) present in every case of the disease and (2) isolated from the host with the disease and grown in pure culture, (3) the specific disease must be reproduced when a pure culture of the microbe is inoculated into a healthy susceptible host, and (4) the pathogen must be recoverable from the experimentally infected host. In 1892, when various bacteria had already been identified, Dimitrii Ivanovsky demonstrated that an infectious agent underlying tobacco mosaic virus disease was small enough to pass through a filter that trapped all known pathogens. Based on this method, which has to be considered cutting edge for that period, a human illness with an unknown etiology was believed to be caused by an unknown blood poison: multiple sclerosis (MS). The Greek word for poison is virus, and when the invention of the electron microscope allowed the visualization of viruses in the 1930s, modern virology was born.

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Little time passed before viral pathogens were implicated in the pathogenesis of MS. In the mid-1930s, the first instances of murine central nervous system (CNS) disease caused by the neurotropic ribonucleic acid picornavirus Theiler murine encephalomyelitis virus were reported. Theiler murine encephalomyelitis has since served as a model to explain infectious and parainfectious mechanisms underlying CNS demyelination. The advent of modern epidemiology and genetics sparked further enthusiasm for the infectious hypothesis of MS. Geographical and seasonal variations in the incidence and prevalence of MS and migration studies implicated exposure to environmental factors, possibly viruses, in MS pathogenesis. A concordance rate for MS of only 30% among monozygotic twins confirmed an etiologic role for nongenomic, environmental factors. Technological advances have led to an inflation of organisms associated with MS. However, despite the development of new, more sensitive techniques that detect growing numbers of viruses, not a single candidate pathogen has gained acceptance as the causal agent in MS. Interferon beta, an approved therapy for MS, was originally proposed as a cytokine capable of increasing the resistance of host tissues against viral infections. To date, viral inhibition as one of the underlying mechanisms of interferon beta in MS has not been supported by any scientific data.

Immediately after viruses were discovered, their relevance in CNS demyelinating diseases was disputed. By inducing demyelination through immunization with CNS homogenate in the absence of any demonstrable pathogen, Rivers et al[2] established the autoimmune hypothesis of MS in 1933. Experimental autoimmune encephalomyelitis has been the archetypal model for MS and has allowed neuroimmunologists to study basic mechanisms underlying CNS autoimmunity.

The partial response of patients with MS to immunosuppressive and immunomodulatory therapy is cited as evidence supporting an autoimmune etiology for MS. However, the major criticism of the autoimmune hypothesis is similar to that of the infectious hypothesis: an autoantigen(s) specific to and causative for MS has never been identified.

The absence of identifiable pathogens and autoantigens continues to be a dilemma. Results of whole genome screens of multiplex MS families have also not provided a conclusive answer and consistently identified genes that may be relevant in both host defense and autoimmunity. Thus, both hypotheses of MS have coexisted, and concepts have emerged that explain one (the autoimmune hypothesis) with the other (the infectious hypothesis).

An infection with a neurotropic virus may cause direct injury to the CNS (**Figure**). Disruption of the blood-brain barrier may then facilitate the release of CNS autoantigen(s) into the blood compartment (Figure). These autoantigens, which are normally not readily exposed in an immunogenic manner, may now be recognized by the systemic immune system and lead to expansion of myelin-specific lymphocytes. In the experimental autoimmune encephalomyelitis and Theiler murine encephalomyelitis models of MS, it was observed that T-cell responses to a dominant determinant of a myelin autoantigen or a pathogen may be followed by recruitment of inflammatory T cells that recognize other, previously cryptic autoantigenic epitopes. This epitope spreading may contribute to disease relapses in MS.

Another concept, molecular mimicry, proposes that a pathogen's protein(s) has structural homology with myelin protein antigen(s) yet is different enough to be recognized as foreign by the host's immune system (Figure). T cells and/or B cells, activated by the infectious agent in the periphery, may then enter the CNS, induce major histocompatibility complex class II expression, and facilitate recognition of myelin antigens and inflammation (Figure).

In a third model, proteins produced by bacteria or viruses are potent activators of CD4+ T cells. These su-

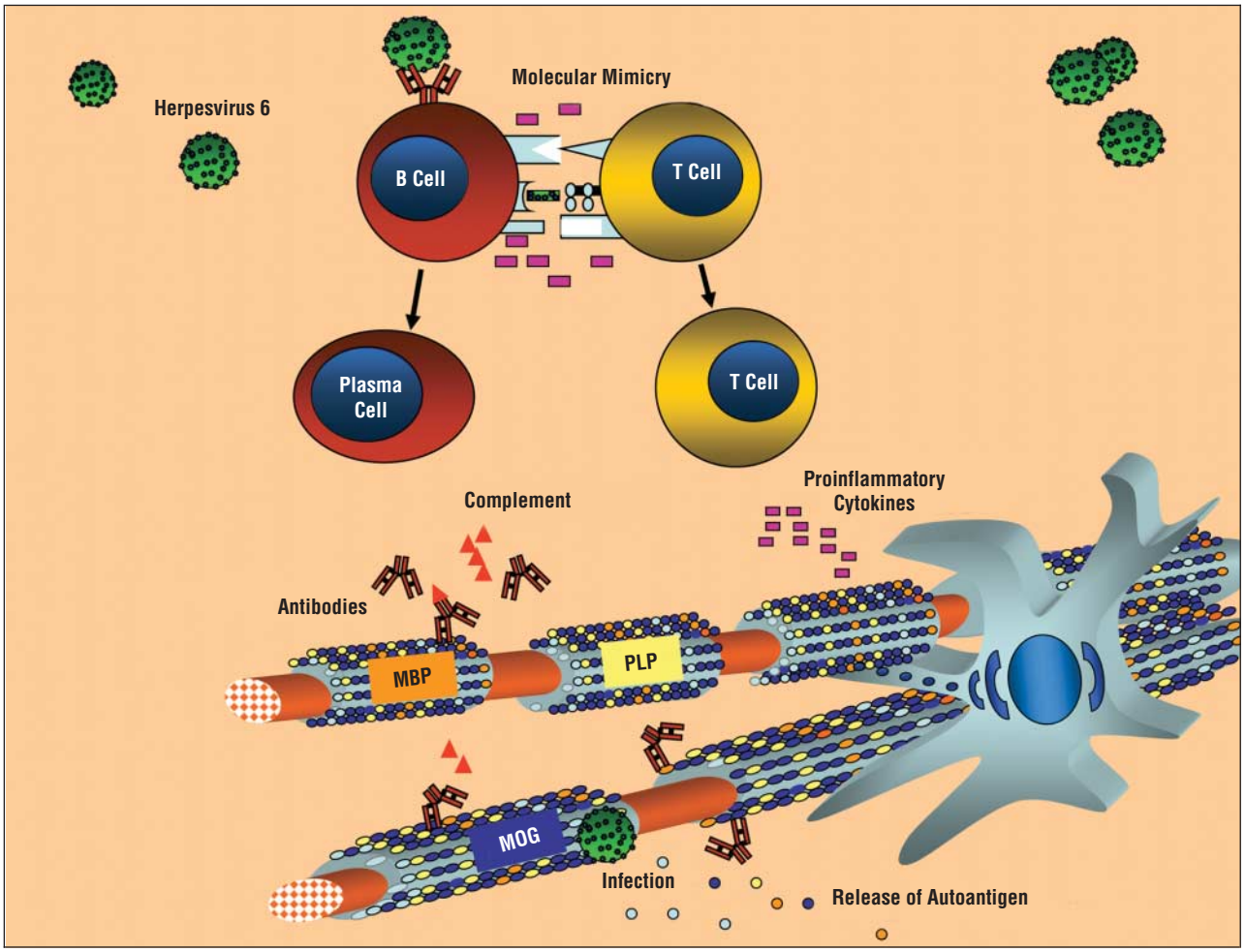

Figure. An infection with a neurotropic virus may cause direct injury to oligodendrocytes and axons. Ongoing inflammation may then result in disruption of the blood-brain barrier, which may ultimately facilitate the release of central nervous system autoantigen(s) into the blood compartment. These autoantigens, which are normally not readily exposed in an immunogenic manner, may now be recognized by the systemic immune system and lead to expansion of myelin-specific lymphocytes. Another concept, molecular mimicry, proposes that a pathogen's protein(s) has structural homology with myelin protein antigen(s) yet is different enough to be recognized as foreign by the host's immune system. T cells and/or B cells, activated by the infectious agent in the periphery, may then enter the central nervous system and facilitate the recognition of myelin antigens. MBP indicates myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; and PLP, proteolipid protein.

perantigens are not processed for antigen presentation but instead bind directly to the MHC class II/CD4+ T-cell complex outside the antigen-binding groove (Figure). A very rapid and potent activation of CD4+ T cells may then contribute to chronic autoimmune diseases.

Herpesvirus 6 (HHV-6) is a DNA virus and a member of the β herpesvirus subfamily of *Herpesviridae*. Two variants exist: HHV-6B accounts for most human infections, whereas the HHV-6A variant has not yet been associated with human disease. Although the worldwide seroprevalence is nearly 100%, there are several aspects of HHV-6 that make it an interesting candidate pathogen in MS pathogenesis: (1) the initial infection typically occurs during infancy or early childhood; (2) HHV-6 is very leukotropic and neurotropic and may persist within the CNS in a latent stage for a lifetime; and (3) reactivation of an HHV-6 infection may occur in the setting of another microbial infection or other physiologic stressors. Reports regarding the prevalence, distribution, and specificity of HHV-6 in patients with MS have been conflicting. Immune responses against the virus or the viral genome have been detected in several tissues. In serum, anti–HHV-6 immunoglobulin M responses were reported to be significantly increased compared with controls.[3,4] Patients with MS who had HHV-6 viremia were reportedly younger and had a significantly shorter disease duration than patients with MS who did not have viremia, suggesting active or reactivated HHV-6 as causative in MS.[5] Herpesvirus 6 DNA was also detected in the cerebrospinal fluid of some patients with MS.[6] Recently, serum levels of soluble CD46, the cellular receptor for HHV-6, were found to be significantly elevated in the cerebrospinal fluid and serum of patients with MS compared with controls.[7] Nuclear staining of oligodendrocytes for HHV-6 was detected by immunohistochemistry exclusively in MS brain specimens, with the highest prevalence within plaque regions.[8] Another report demonstrated HHV-6 in microglia, lymphocytes, and possibly oligodendrocytes in the majority of patients with MS and HHV-6 leukoencephalitis but not in controls.[5] Herpesvirus 6 DNA was detected significantly more frequently in MS plaques than in normal-appearing white matter or control CNS tissue. In situ polymerase chain reaction revealed numerous oligodendrocytes, lymphocytes, and mi-

croglia containing HHV-6 genome within acute MS lesions.[9] Herpesvirus 6 DNA has also been demonstrated in urine and saliva samples of patients with MS who had some pathognomonic specificity.[10,11]

Despite the multitude of reports implicating HHV-6 in CNS autoimmune disease, controversy exists that has led to some skepticism among neuroimmunologists. Numerous reports have contradicted some of the findings previously mentioned. In one, anti–HHV-6 immunoglobulin M antibodies were detected only in a small minority of patients with MS, whereas immunoglobulin G antibodies were significantly more prevalent.[12] Furthermore, antigen-specific T-cell responses to several HHV-6 antigens did not differ between patients with MS and healthy controls. Extremely sensitive polymerase chain reaction–based detection methods often do not provide conclusive answers either: one study demonstrated a similar HHV-6 prevalence in MS brain tissue to that of neurologic controls.[8] Even more surprisingly, various studies failed to detect HHV-6 in any tissue from patients with MS.[13-15] Perhaps the major shortcoming of existing studies is that viremia and virus-specific immune responses have rarely been correlated longitudinally with clinical or neuroimaging data. One recent study examined the relationship between HHV-6 DNA and RNA in the blood and MS disease activity monitored by magnetic resonance imaging in relapsing-remitting and secondary progressive patients with MS. Interestingly, HHV-6 reactivation correlated with the number of gadolinium-enhancing lesions found on magnetic resonance imaging.[16]

In the present study, Álvarez-Lafuente et al[17] enrolled a total of 105 patients with relapsing-remitting MS and 49 healthy controls to evaluate the prevalence of HHV-6 in serum and blood. Their experimental approach is interesting. Rather than limiting itself to the detection of HHV-6, this study tries to correlate MS and HHV-6 disease activity. Among patients with relapsing-remitting MS who suffered from active HHV-6 infection, only the A variant was detected. The HHV-6 viral load was higher in patients with MS who had evidence of active viral replication and who suffered from an acute attack than in those in remission. Patients with latent infection were seropositive for both HHV-6 variants, whereas only variant B was identifiable in healthy controls. The differential expression of 3 HHV-6 gene transcripts confirmed the occurrence of HHV-6 active infection in patients with MS.

The correlation of an active infection with HHV-6 variant A and MS is certainly an interesting observation. As the HHV-6A variant has not yet been associated with another human disease, the present finding would suggest it to be pathognomonic for MS. Whether HHV-6 causes direct tissue damage, or whether concepts like epitope spreading, molecular mimicry, and superantigens apply, will need to be clarified.

It is surprising that no differences in DNA prevalence or in viral load of Epstein-Barr virus between patients with MS who are in remission and those during a disease relapse were demonstrated. Because Epstein-Barr virus has been associated with MS in numerous previous studies, these results are slightly disturbing and may represent methodological differences.

Álvarez-Lafuente and colleagues combined sophisticated technology and an interesting scientific question to provide some thought-provoking results. Not all questions regarding the role of HHV-6 in MS have been answered, and several requirements for proving Koch's postulate for HHV-6 are certainly still missing. In the meantime, the search for the invisible pathogens will keep us intrigued.

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