EDITORIAL

Progressive Multifocal Leukoencephalopathy and Natalizumab — Unforeseen Consequences

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In this issue of the *Journal*, there are reports describing in detail three patients in whom progressive multifocal leukoencephalopathy (PML) developed during treatment with natalizumab, a humanized monoclonal antibody against α_4 integrins.[1-3] These patients were among 3000 who had participated in clinical trials of natalizumab for the treatment of multiple sclerosis or Crohn's disease. PML is a deadly opportunistic infection of the central nervous system (CNS) for which there is no specific treatment. It is caused by reactivation of a clinically latent JC polyomavirus infection. This virus infects and destroys oligodendrocytes, leading to multifocal areas of demyelination and associated neurologic dysfunction. The occurrence of PML in this setting was totally unexpected, since it almost invariably occurs in the context of profoundly impaired cell-mediated immunity in patients with AIDS or leukemia or in organ-transplant recipients.

In retrospect, can we retrace the events that led to the surprising development of PML in these three patients? Seropositivity rates for JC virus, the etiologic agent of PML, increase with age and vary in different populations. After infection, the virus remains quiescent in the kidneys and in lymphoid organs of people with immunocompetence. The virus is often present in the urine but is generally not found in the blood. However, JC viremia can be detected in persons with immunosuppression, and hematogenous dissemination is the likely route of entry into the CNS.[4]

Since the authors of the present reports did not provide data on the serologic status of JC virus for the patients, we can only assume that the patients had been infected in childhood. If this is the case, what role did the multiple medications taken by these persons play in the reactivation of JC virus, which eventually led to PML? Retrospective analysis of serum samples that were obtained between 1999 and 2003 from the patient with Crohn's disease provides an important answer: JC virus became detectable only in May 2003, after three injections of natalizumab monotherapy, two months before the patient was admitted to the hospital. Moreover, the serum viral load increased by a factor of 10 after two additional injections.

Therefore, it appears likely that natalizumab, by preventing normal trafficking of lymphocytes, led to unbridled JC virus replication in this patient. Consistent with this scenario, inflammatory infiltrates were conspicuously absent from the brain lesions. Indeed, the cellular immune response, principally mediated by CD8+ cytotoxic T lymphocytes, has been shown to play a major role in the containment of JC virus.[5,6] In the patients with multiple sclerosis, the presence of JC viremia was not tested retrospectively before the onset of neurologic symptoms of PML, and the role of the concomitant administration of interferon beta-1a in JC virus reactivation remains unknown. However, PML has not been reported previously in association with this disease-modifying agent for multiple sclerosis.

If impaired immune surveillance due to treatment was responsible for the development of PML, from what site or sites did the virus reactivate? The lack of analysis of urine and kidney or lymphoid tissues precludes comment on whether these organs were the epicenter of JC virus dissemination in the body.

Could the virus have already been present in a latent stage in the brain, or did it reactivate from

multiple sites? There are conflicting reports in regard to the detection of JC virus in the CNS and the digestive systems of persons with immunocompetence, and this issue is still unsettled. In the patient with Crohn's disease, however, intestinal samples obtained three years before the development of PML showed no JC virus DNA. In any event, the development of PML in these three patients — in whom the entry of mononuclear cells into the brain, at least in theory, should have been significantly diminished or entirely blocked — suggests that JC virus may enter by other means, perhaps as free virus.

What can we learn from the clinical course of these patients' illnesses? Certain elements of the cases are worthy of comment. Arriving at a diagnosis of PML may be challenging, and mistaking PML for a primary brain tumor or a stroke may occur on occasion. However, the imaging findings of a multifocal process that is limited to the white matter and that exhibits neither mass effect nor enhancement with contrast material should always raise the suspicion of PML. In the absence of a contraindication to lumbar puncture, the demonstration of a positive result on polymerase chain reaction (PCR) for JC virus in the cerebrospinal fluid establishes the diagnosis when coupled with the appropriate clinical and radiologic features.

The classic histopathologic hallmarks of PML include enlarged oligodendroglial nuclei at the border of areas of demyelination; giant, bizarre astrocytes; and lipid-laden macrophages that scavenge myelin debris. In situ hybridization or immunohistochemical staining permits identification of the virus. The pathological findings in one of the patients with multiple sclerosis[1] of massive, coalescent areas of cavitation of the brain are atypical, although not unheard of, in PML.

Since treatment with natalizumab was eventually discontinued in the three patients, how can we explain the differences in their clinical outcomes? In patients with AIDS who have PML and are treated with highly active antiretroviral therapy, recovery of the immune system is associated with increased long-term survival (i.e., more than one year), from just under 10 percent[7] to approximately 50 percent.[8] One may therefore wonder why the two patients who died four[1] and five[3] months after the time of presentation did not stabilize or recover once natalizumab administration had been stopped. By binding to the surface of lymphocytes and monocytes, natalizumab prevents the passage of these cells from the bloodstream into the parenchyma of various organs. Perhaps the fact that as many as 80 percent of the α_4 integrin receptors of peripheral blood lymphocytes remain saturated one month after infusion[9] explains this continuing process, and a biologic effect may be observed up to three months after the administration of natalizumab.[10] Consistent with this hypothesis, approximately three months after discontinuing natalizumab, the surviving patient with multiple sclerosis presented with an immune-reconstitution inflammatory syndrome,[11] which was probably caused by the return of lymphocytes to the CNS and was characterized by contrast enhancement of PML lesions. This inflammatory reaction was associated with a decreased JC viral load in the blood and cerebrospinal fluid as well as transient worsening followed by neurologic improvement. This patient was also the only one who received medications other than corticosteroids, including intravenous immune globulin, cidofovir, and cytarabine. Of these, only cytarabine has been demonstrated to have activity against JC virus in vitro. It is interesting that the breakdown of the blood–brain barrier in this patient was probably instrumental in increasing the intraparenchymal distribution of this drug, which has limited efficacy in the treatment of PML[12,13] due to poor penetration into the CNS.[14] Furthermore, the incidental finding of an asymptomatic PML lesion on magnetic resonance imaging may have led to earlier intervention in this patient's clinical course, as compared with the patients who died from PML. In the latter patients, the disease was probably too advanced once the effect of natalizumab wore off to be contained by lymphocytes capable of returning to the brain parenchyma.

Would it be possible to predict and prevent the occurrence of PML in patients receiving α_4-integrin blockers? Only persons infected with JC virus are at risk for PML, but the rate of seropositivity for this virus is from 50 to 86 percent in healthy adults.[15,16] This is probably explained in part by differences in the sensitivity of the assays used and underscores the need for a highly sensitive, universally accepted enzyme-linked immunosorbent assay.

If past serum samples are available, retrospective measurement by quantified PCR of the JC viral load in the plasma of patients with Crohn's disease and multiple sclerosis who were treated with natalizumab will be necessary to determine the predictive value of this test for the development of PML in patients with these conditions. This information

will probably be equally important with regard to other biologic agents or medications that inhibit lymphocyte migration. The data acquired from such a retrospective analysis would be essential to determine whether it will be possible to fashion preventive strategies against the development of PML in patients treated with natalizumab or similar drugs in the future.

The prospective measurement of the JC viral load in plasma and the preemptive reduction of doses or interruption of treatment if JC virus DNA appears in the blood might actually prevent the development of PML in this setting. By analogy, a similar strategy has been used successfully for the prevention of a nephropathy caused by the JC virus-related BK polyomavirus in kidney-transplant recipients.[17] In addition, analysis of the JC virus regulatory region, which contains determinants of neurotropism and neurovirulence,[18] may provide important insight into the mechanisms of JC virus reactivation in patients treated with novel immunomodulatory medications.

Finally, these observations provide a unique and unexpected window into our understanding of the pathogenesis of PML and force us to reconsider the potential risks associated with inhibition of lymphocyte trafficking. Bad things may happen when rescuers are turned back at the gates.

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