BRIEF REPORT

Progressive Multifocal Leukoencephalopathy in a Patient Treated with Natalizumab

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SUMMARY

We describe the clinical course of a patient with multiple sclerosis in whom progressive multifocal leukoencephalopathy (PML), an opportunistic viral infection of the central nervous system, developed during treatment with interferon beta-1a and a selective adhesion-molecule blocker, natalizumab. The first PML lesion apparent on magnetic resonance imaging was indistinguishable from a multiple sclerosis lesion. Despite treatment with corticosteroids, cidofovir, and intravenous immune globulin, PML progressed rapidly, rendering the patient quadriparetic, globally aphasic, and minimally responsive. Three months after natalizumab therapy was discontinued, changes consistent with an immune-reconstitution inflammatory syndrome developed. The patient was treated with systemic cytarabine, and two months later, his condition had improved.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY (PML) IS A RARE, OLIgodendroglial infection caused by the polyomavirus JC virus. It usually occurs in people infected with the human immunodeficiency virus (HIV), but it has also been reported in immunocompromised patients receiving prolonged treatment with methotrexate, cyclophosphamide, and azathioprine. PML has not been reported in persons with multiple sclerosis, despite the frequent use of these medications to treat the disease.

We describe the clinical course of a patient with multiple sclerosis in whom PML developed during treatment with interferon beta-1a (Avonex, Biogen Idec) and natalizumab (Tysabri, Biogen Idec and Elan), a monoclonal antibody against α_4 integrins. Despite the discontinuation of these medications, his PML progressed rapidly. An immune-reconstitution inflammatory syndrome developed three months after the cessation of natalizumab therapy, and the patient became bedridden and minimally responsive. Treatment with intravenous cytarabine was begun, and shortly thereafter, his condition improved. The reasons for his clinical deterioration and recovery are not clear.

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CASE REPORT

In 1983, a 23-year-old right-handed man had a month-long episode of right hemianesthesia, his first symptom of what proved to be relapsing–remitting multiple sclerosis. He had a second attack in 1989 and had two or three attacks per year between 1989 and 1998. His medical history was also notable for the Ramsay Hunt syndrome with auricular zoster in 1998, a malignant melanoma excised from his back with negative margins in 1996, and a cleft lip and palate. A sister also had relapsing–remitting multiple sclerosis.

He started receiving weekly intramuscular injections of interferon beta-1a in 1998 (Fig. 1). The frequency of relapses decreased to one per year until 2001. From 2001 through 2002 he had three exacerbations, prompting his enrollment in a double-blind, randomized, placebo-controlled trial of 300 mg of natalizumab every four weeks plus interferon beta-1a as compared with a placebo infusion plus interferon beta-1a. At entry into the study in October 2002, he had an old left afferent pupillary deficit, mild right lateral rectus palsy, right-sided lower-motor-neuron facial paresis, mild ataxia, a score on the Kurtzke Expanded Disability Status Scale of 2 (scores can range from 0 to 10, with higher scores indicating more severe disease), and evidence of focal, nonenhancing white-matter lesions on T_2-weighted magnetic resonance imaging (MRI) characteristic of multiple sclerosis. During the next two years he had no further relapses. T_2-weighted MRI of the brain, performed as part of the study protocol in October 2003, showed multiple small, nonenhancing periventricular and subcortical hyperintensities consistent with the presence of multiple sclerosis. But in October 2004, in addition to a small, new, enhancing periventricular lesion typical of multiple sclerosis (not shown), a new nonenhancing lesion of the right frontal lobe appeared on another MRI scan obtained as part of the protocol (Fig. 2A).

In November 2004, the patient's physician observed uncharacteristic, inappropriate behavior during a routine study visit. In mid-December, the patient told his family and friends that he was having difficulty with attention and concentration. Progressive left hemiparesis, dysarthria, and cognitive impairment subsequently developed. MRI of the brain showed new, extensive abnormalities, including a large hyperintense lesion of the right frontal lobe, bilateral subinsular white-matter lesions that spared the cortex, and scattered lesions in the white matter, deep gray matter, and brain stem, with a few punctate foci of enhancement consistent with the presence of noninflammatory PML[1] (Fig. 2B). After receiving 28 infusions, the last in mid-December 2004, the patient stopped taking the study drug, which was revealed to be natalizumab.

The patient was not classically immunocompromised at clinical presentation: he had no known risk factors for HIV infection, serologic analysis for HIV was twice negative, and the total leukocyte count (8.6×10^3 per cubic millimeter) and values for lymphocyte subgroups were normal (CD4:CD8 ratio, 1.1; CD4 T-cell count, 637 per cubic millimeter; and CD8 T-cell count, 564 per cubic millimeter). Analysis of cerebrospinal fluid in early February showed no white cells and 88 red cells per cubic millimeter, normal cytologic findings, and normal concentrations of both total protein (41 mg per deciliter) and glucose (62 mg per deciliter [3.4 mmol per liter]). The IgG index (a measure of the IgG production in the cerebrospinal fluid) was elevated (0.7), and two oligoclonal bands were seen. JC virus DNA was detected by the polymerase chain reaction (PCR)[2] in the serum (2500 copies per milliliter), peripheral-blood mononuclear cells (225 copies per milliliter), and cerebrospinal fluid (6050 copies per milliliter). Biopsy of the right frontal lobe showed abundant areas of astrogliosis and microgliosis in the deep layers of cortical gray matter, with underlying white matter showing demyelination, dense infiltration of macrophages, and sparse lymphocytes. Scattered enlarged oligodendrocytes contained intranuclear inclusions positive for papovavirus (Fig. 3). In situ hybridization showed JC virus but no evidence of herpes simplex virus or cytomegalovirus. A workup for cancer, including computed tomography (CT) of the chest, abdomen, and pelvis and whole-body positron-emission tomography, showed no masses and no areas of increased metabolism. Positron-emission tomography did show decreased cortical uptake of fludeoxyglucose F 18 within the right frontal lobe, a finding consistent with necrosis.

During the next three weeks, left hemiplegia, left-sided neglect, left hemianesthesia, apraxia of the right arm, and nonfluent aphasia developed and dysarthria worsened despite intravenous treatment with high-dose methylprednisolone. Intravenous treatment with cidofovir (5 mg per kilogram of body weight every two weeks) was initiated.

Eight days later, global aphasia, incontinence, stooped posture, and truncal instability developed. Repeated analysis of cerebrospinal fluid showed a mild pleocytosis and hemorrhage: an elevated protein concentration (58 mg per deciliter), 2 white cells and 324 red cells in the second tube obtained, and 6 white cells (30 percent neutrophils, 55 percent lymphocytes, 4 percent reactive lymphocytes, and 11 percent monocytoid cells) and 913 red cells in the subsequent tube. JC virus DNA was undetectable in peripheral-blood mononuclear cells and plasma but remained present in the cerebrospinal

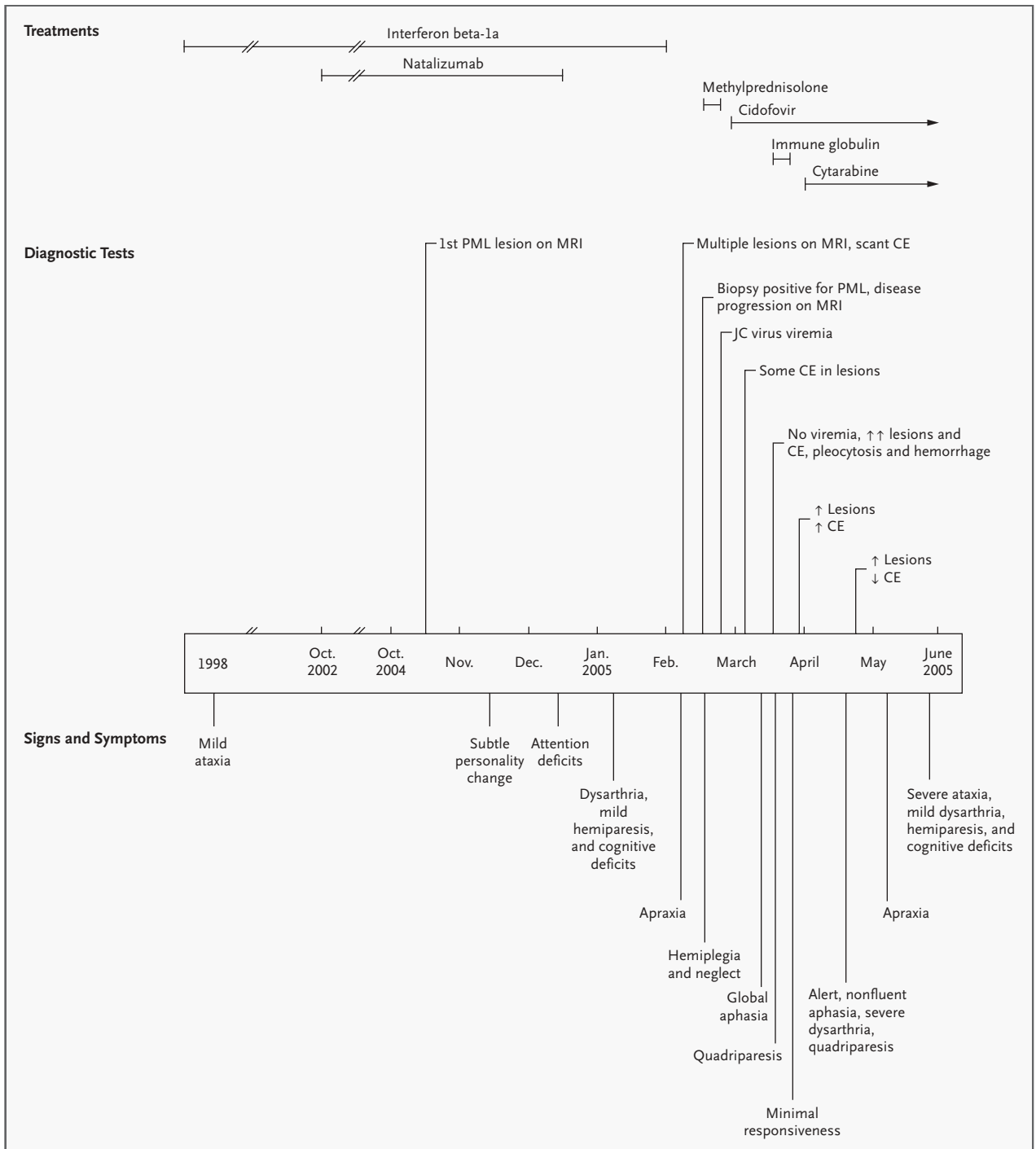

Figure 1. Summary of the Patient's Clinical Course, Treatments, and Results of Laboratory Tests.

CE denotes contrast enhancement. One upward-pointing arrow indicates a moderate increase, two upward-pointing arrows a substantial increase, and one downward-pointing arrow a moderate decrease.

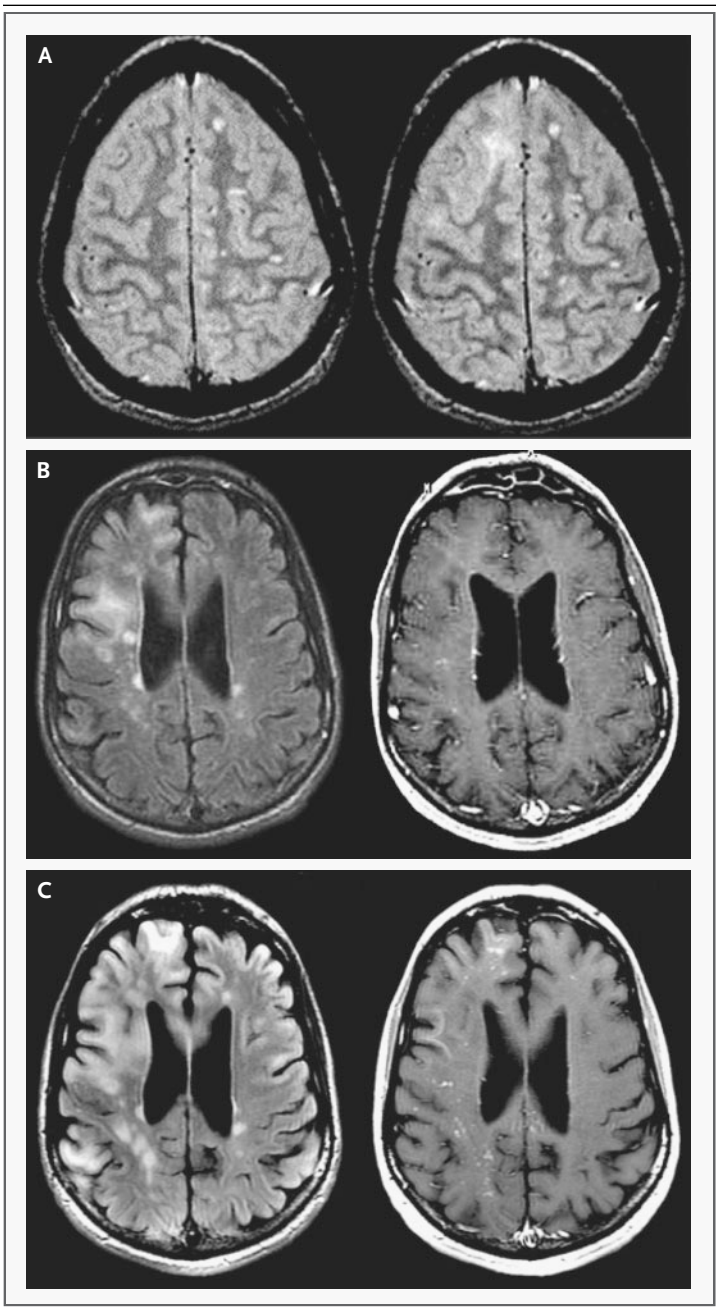

Figure 2. Progression of Abnormalities on MRI.

Panel A shows images obtained before the development of PML-related symptoms. An axial T_2-weighted MRI obtained in October 2003 (left-hand side) shows multiple small focal lesions in the white matter consistent with the presence of multiple sclerosis. In October 2004 (right-hand side), a large, new, ill-defined lesion is seen in the right frontal lobe, which will later prove to be PML. In early February 2005 (Panel B), axial fluid-attenuated inversion recovery MRI (left-hand side) shows more extensive disease in the right frontal white matter, with cortical sparing and several scattered lesions. After the addition of intravenous contrast medium (right-hand side), a few small foci of enhancement are apparent in the right hemisphere. In late March 2005 (Panel C), axial fluid-attenuated inversion recovery MRI (left-hand side) shows dramatic progression, especially in the right hemisphere, with lesions now extending into the anterior corpus callosum. After the addition of intravenous contrast medium (right-hand side), there is a substantial increase in the foci of enhancement.

fluid (2245 copies per milliliter). PCR of cerebrospinal fluid for herpes simplex virus, human herpesvirus 6, varicella–zoster virus, Epstein–Barr virus, and enteroviruses was negative, as were the results of Gram's staining, bacterial culture, cryptococcal staining, staining for acid-fast bacilli, and serologic analysis for Lyme disease. CT of the head showed no evidence of hemorrhage. MRI of the brain five weeks later (Fig. 2C) showed marked progression of the white- and gray-matter lesions and extensive foci of enhancement, particularly in the right hemisphere, findings consistent with inflammation.

The patient's hospital course was further complicated by methicillin-resistant *Staphylococcus aureus* bacteremia, urosepsis, upper gastrointestinal bleeding, elevated concentrations of serum aminotransferases, transient hyponatremia, and transient lymphopenia. The nadir absolute lymphocyte count was 647 cells per cubic millimeter, with 188 CD4+ T cells per cubic millimeter, 214 CD8+ T cells per cubic millimeter, and a CD4:CD8 ratio of 0.9.

His condition continued to deteriorate, despite the administration of three infusions of cidofovir over a period of eight weeks and a five-day course of intravenous immune globulin (2 g per kilogram per day). Left hemiplegia, anesthesia, and neglect were now accompanied by right hemiparesis and apraxia, nonfluent aphasia, severe cognitive impairment, and a fluctuating level of alertness, rendering the patient bedridden, mute, and almost completely noncommunicative. Electroencephalography at this time showed diffuse slowing and bilateral periodic epileptiform discharges that did not respond to treatment with intravenous benzodiazepam.

His treating physicians began intravenous treatment with cytarabine (2 mg per kilogram per day for five days) in early April. This caused pancytopenia, requiring the administration of erythropoietin and granulocyte colony-stimulating factor, and fever; the latter resolved within 12 hours after empirical antibiotic treatment.

Unexpectedly, the patient began talking two weeks after the initiation of cytarabine therapy. At the time of the most recent follow-up assessment, he continued to show neurologic improvement. After one month of cytarabine therapy, his right-sided weakness and left-sided sensory loss resolved, and his left hemiplegia, neglect, aphasia, and dysarthria began to improve. He still had severe deficits, including dysarthria, spastic left hemiparesis, cognitive impairment, and parkinsonism. He required the assistance of two persons to move from a bed to a chair. MRI of the brain obtained three weeks after treatment with cytarabine was begun showed further progression of disease in the left cerebellar white matter, right external and internal capsule, and frontal lobes bilaterally. The only detectable improvement was a slight decrease in the amount of contrast enhancement.

A second course of cytarabine was given four weeks after the first, without any complications. By the end of May 2005, the patient was starting to walk and was having meaningful conversations regarding the reasons for his clinical deterioration. He still had disabling ataxia, cognitive impairment, mild neglect, and mild left hemiparesis.

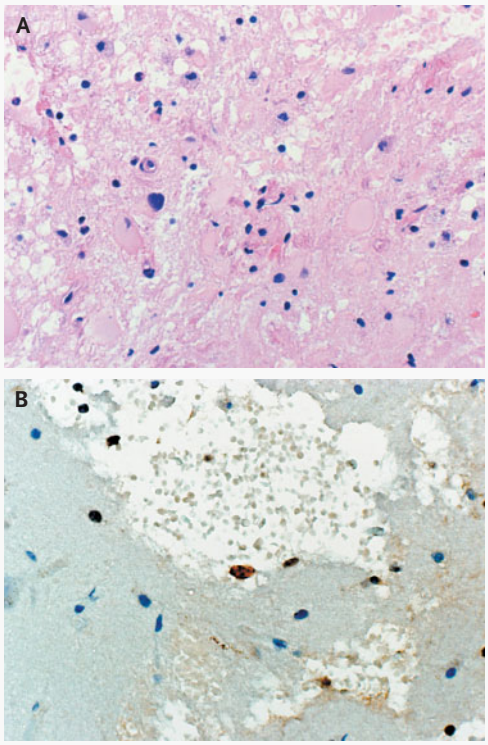

Figure 3. Brain-Biopsy Specimen.
Panel A shows a focus of demyelination (hematoxylin and eosin), and Panel B immunohistochemical staining for papovavirus.

DISCUSSION

Our patient is one of three patients in whom rapidly progressive PML has been shown to develop during clinical trials of natalizumab, a selective adhesion-molecule blocker, to treat relapsing–remitting multiple sclerosis or Crohn's disease.[3-5] Elsewhere in this issue of the *Journal*, Kleinschmidt-DeMasters and Tyler describe a second patient with multiple sclerosis who received combination treatment with natalizumab and interferon beta-1a[3] and Van Assche et al. describe a patient with Crohn's disease who received natalizumab alone.[4]

Our patient's condition worsened after the cessation of natalizumab therapy despite treatment with cidofovir, corticosteroids, and intravenous immune globulin, but his condition improved after the institution of systemic cytarabine therapy. His brain biopsy showed typical noninflammatory PML; however, three months after the cessation of natalizumab, what we believe to be an immune-reconstitution inflammatory syndrome developed that was characterized by widespread inflammation of the central nervous system, as shown by extensive enhancement on MRI and microscopic hemorrhages. Other remarkable features of the case include JC virus viremia and MRI evidence of PML one month before symptoms developed.

JC virus is a ubiquitous infection acquired in childhood that remains dormant in bone marrow, kidney epithelia, and spleen. Antibodies against JC virus are detectable in at least 80 percent of adults.[6] However, humoral immunity is insufficient to prevent the spread of the virus to the central nervous system. Intermittent reactivation, with shedding of live virus in the urine, has been well documented in cross-sectional studies of healthy adults and pregnant women, but this phenomenon is poorly understood. Spread of the virus to the central nervous system and the subsequent development of PML occur in immunocompromised persons — most commonly those infected with HIV, but also in some patients with lymphoma, sarcoidosis, and medication-induced immunosuppression. JC virus can enter the central nervous system directly during periods of viremia, such as those occurring during prolonged immunosuppression. Eighty

to 90 percent of patients with PML but not HIV infection die within one year.[7]

Natalizumab is highly effective at preventing recurrent inflammation in patients with multiple sclerosis.[8] Natalizumab binds to and blocks the function of α_4 integrins, adhesion molecules that promote the migration of lymphocytes into various organs, including the brain[9] and kidneys.[10] In patients with multiple sclerosis, natalizumab's most striking effect is the reduction of both contrast-enhancing lesions on MRI and clinical relapses.[8]

How natalizumab therapy alone or in combination with other immune-altering therapies could lead to JC virus viremia and PML is unknown. We speculate that the reactivation of the virus cannot be suppressed until the effects of natalizumab wear off. In our patient, JC virus viremia ended three months after treatment with natalizumab was stopped, and the biologic effects of natalizumab have been shown to wear off after about three months.[11]

Three months after natalizumab therapy was stopped, an inflammatory reaction developed in our patient's brain. In HIV-infected patients, as in our patient, inflammatory reactions against PML are a manifestation of the immune-reconstitution inflammatory syndrome and are associated with clinical deterioration and increases in the size of high signal lesions on T_2-weighted MRI but more favorable outcomes than in noninflammatory PML.[12,13] However, patients can die during the course of the immune-reconstitution inflammatory syndrome,[13] as our patient almost did, and how best to manage the JC virus infection and this inflammatory phase of PML is unknown.

Cidofovir, an antiviral agent, has been used with anecdotal success in the treatment of HIV-associated PML.[14,15] However, in vitro, cidofovir fails to kill glial cells infected with JC virus,[16] and there are no controlled studies to support its use. After three courses of cidofovir, our patient's condition continued to deteriorate.

Cytarabine kills JC virus in vitro.[16] This observation led to a randomized, controlled trial of the drug in HIV-infected patients with PML, which failed to show efficacy.[17] However, the penetration of cytarabine into the central nervous system is poor, and only one patient in this trial had contrast enhancement on MRI.[18] We chose to administer cytarabine to our patient, given the failure of cidofovir and the lack of other options, and subsequently, his condition improved. The reasons for this improvement are not clear. It is possible that the extensive breakdown of his blood–brain barrier improved penetration of cytarabine into the central nervous system, aiding in the clearance of the virus, or that its strong myelosuppressive properties curbed the inflammatory response. Alternatively, the improvement may have been due solely to clearance of the virus by the patient's reconstituted immune system.

In our patient, the first PML lesion — a frontal-lobe lesion that was indistinguishable from a multiple sclerosis lesion — was visible on neuroimaging studies two months before obvious neurologic deficits developed. Although this may be due to the relatively subtle deficits that would be expected as a result of a lesion in this area, it suggests that more frequent MRI monitoring of patients who receive natalizumab may be warranted. The appearance of lesions, particularly in or abutting the gray matter, should increase clinical suspicion of PML. Monitoring for JC virus viremia may also be useful in such patients. Our case report suggests that some degree of recovery from natalizumab-associated PML is possible.

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