

BRIEF REPORT

Progressive Multifocal Leukoencephalopathy after Natalizumab Therapy for Crohn's Disease

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SUMMARY

The prior diagnosis of fatal astrocytoma in a 60-year-old man with Crohn's disease treated with natalizumab, a monoclonal antibody against α_4 integrins, was reclassified as JC virus–related progressive multifocal leukoencephalopathy (PML). Analysis of frozen serum samples showed that JC virus DNA had appeared in the serum three months after the initiation of open-label natalizumab monotherapy and two months before the appearance of symptomatic PML. There was staining of the brain lesion for polyomavirus. This case report, along with two others, suggests that anti- α_4 -integrin therapy can result in JC virus–induced PML.

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NATALIZUMAB HAS GREAT THERAPEUTIC POTENTIAL IN BOTH MULTIPLE sclerosis and inflammatory bowel disease.¹⁻³ Two cases of progressive multifocal leukoencephalopathy (PML) have recently been reported in patients with multiple sclerosis who were treated with a humanized monoclonal antibody against α_4 integrins, natalizumab (Tysabri, Elan and Biogen Idec), in combination with interferon beta-1a (Avonex, Biogen Idec).⁴ One of these cases is described elsewhere in this issue of the *Journal*.⁵ We report a third case of PML — this one in a patient with Crohn's disease who received 300 mg of open-label natalizumab intravenously every four weeks as part of a clinical trial. PML is an opportunistic, infectious, demyelinating brain disorder associated with impaired T-cell function. The relationship between natalizumab therapy and PML in our patient is clearly illustrated by the gradual increase in the number of copies of JC virus in the blood during monotherapy in the months preceding the development of fatal PML.

CASE REPORT

A 60-year-old patient with long-standing ileal Crohn's disease presented to the emergency unit with severe confusion and disorientation on July 3, 2003. Treatment with natalizumab, a humanized monoclonal antibody against α_4 integrins, had been initiated in March 2002. He had initially received three monthly infusions of 300 mg intravenously during the Evaluation of Natalizumab as Continuous Therapy 1 (ENACT-1) trial, followed by treatment with placebo for nine months in the ENACT-2 trial. Open-label natalizumab at a dose of 300 mg given intravenously every four weeks was then resumed in

February 2003 for a relapse of Crohn's disease. The patient received five doses of the drug before he was admitted. He had been treated with multiple therapies during that time, including azathioprine (75 to 150 mg given daily), but this treatment had been discontinued eight months before admission because of refractory anemia with low platelet counts and lymphopenia.

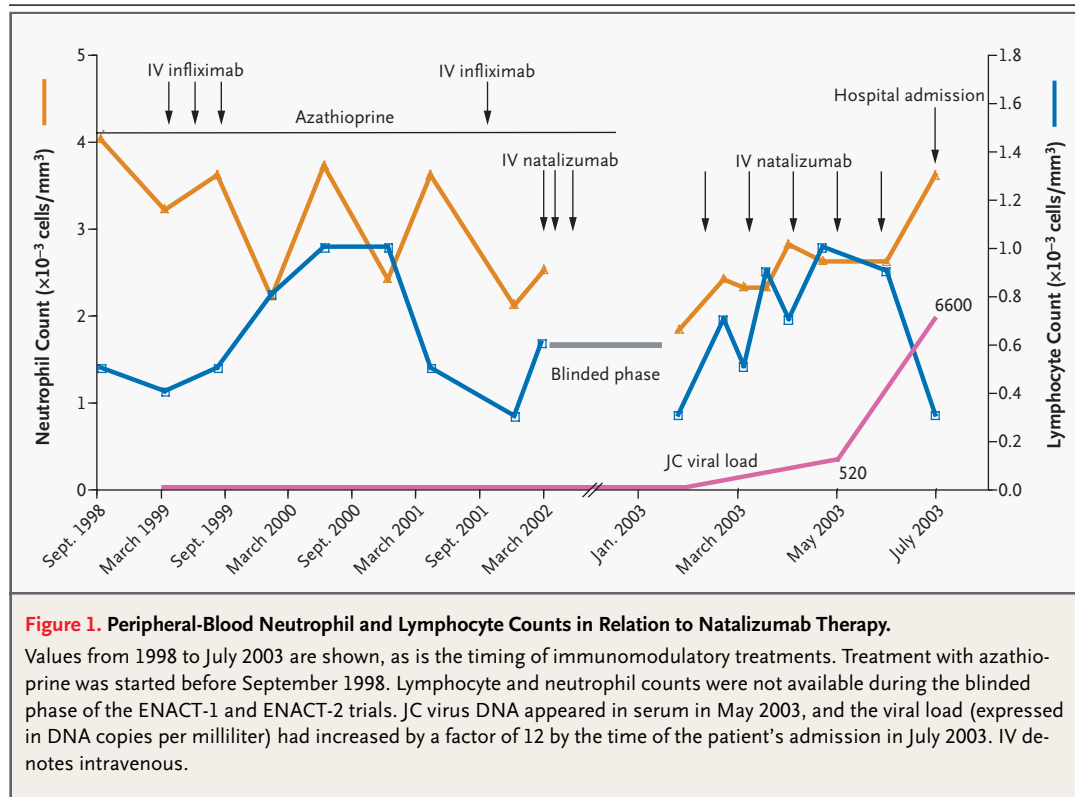
Since 1996, the patient had had intermittent signs of deficient hematopoiesis, with lymphopenia and anemia predominating, regardless of ongoing therapy (Fig. 1). A bone marrow smear had shown pancytopenia despite active hematopoiesis, which was interpreted as reflecting chronic inflammatory disease. Since the diagnosis of Crohn's disease 28 years earlier, the patient had been treated with azathioprine, antibiotics, budesonide, and infliximab (with the last infusion given 20 months before admission). Segmental ileal resection had been performed eight and three years earlier.

On admission, the patient was found to be mentally slow, albeit with normal arousal (Mini-Mental State examination score of 29; the highest score is 30). No focal signs were found on neurologic examination. General physical features were also un-

remarkable, with no fever or abdominal tenderness and with normal vital signs. Routine hematologic and biochemical measurements showed mild iron-deficiency anemia (hemoglobin, 10.7 g per deciliter) with a normal white-cell count and a platelet count of 121,000 per cubic millimeter, a blood glucose level of 150 mg per deciliter (8.3 mmol per liter), a low serum potassium level (3.11 mmol per liter), and a low phosphate level (0.64 mg per deciliter [0.21 mmol per liter]), with otherwise normal serum levels of electrolytes. Levels of C-reactive protein were normal, as were the findings on electrocardiography and chest radiography.

A computed tomographic scan of the brain showed a nonenhancing hypodense lesion in the right frontal lobe. Both T₂-weighted magnetic resonance imaging (MRI) scans and MRI scans obtained with fluid-attenuated inversion recovery revealed hyperintense nonenhancing lesions in the right frontal lobe and in the left frontal and right temporal lobes (Fig. 2).

Because of progressive deterioration in the patient's condition, the decision to perform surgery was made quickly and a spinal tap was not performed. Trephination was performed, with partial



resection of the right frontal lesion. Histologic examination showed that the resected lesion mainly contained abnormalities in the white matter, consisting of a mixture of astrocytes with very large and atypical nuclei, lymphocytes, and foamy macrophages (Fig. 3A). Because of the large frontal lesion, the atypical aspect of the nuclei, and the increase in the Ki67-MIB1 proliferation index (± 15 percent), a diagnosis of astrocytoma of World Health Organization grade III was made.

The postoperative period was characterized by prolonged confusion and somnolence and seizures, treated with phenytoin. After a temporary improvement in the patient's condition, somnolence and confusion again worsened. MRI performed six weeks after surgery showed enlargement of the lesions in the right temporal and left frontal lobes and mainly postoperative changes in the right frontal lobe (shown in Fig. 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). Treatment with corticosteroids was initiated, and radiotherapy was planned. However, the patient's condition deteriorated rapidly, and he died three months after the start of corticosteroid therapy, in December 2003. An autopsy was not performed. At its onset, the neurologic syndrome was reported to the manufacturer of natalizumab and to the local institutional review board as a serious adverse event related to therapy.

On March 1, 2005, all investigational and commercial administrations of natalizumab were halted by Elan and Biogen Idec, owing to the occurrence of PML in two patients with multiple sclerosis who had received this drug in combination with interferon beta-1a.⁴ This announcement prompted us to reexamine our patient's course, in agreement with Elan and Biogen Idec.

METHODS AND RESULTS

DIAGNOSTIC SPECIMENS

Formalin-fixed and paraffin-embedded tissue was available from the resected brain lesion and from colonic biopsy specimens obtained before the treatment with natalizumab was begun. Also, fresh-frozen surgical samples from a previous ileocolonic resection had been stored at -70°C . Serum samples had been collected and stored at -70°C at regular intervals from 1999 until the detection of the brain lesions, as part of a prospective serum bank for patients with inflammatory bowel disease at our institution. Written informed consent for these collections had been obtained. Samples of cerebrospinal fluid and urine were not available.

PATHOLOGICAL FINDINGS

Reexamination of the brain specimens revealed some oligodendrocyte nuclei with a ground-glass

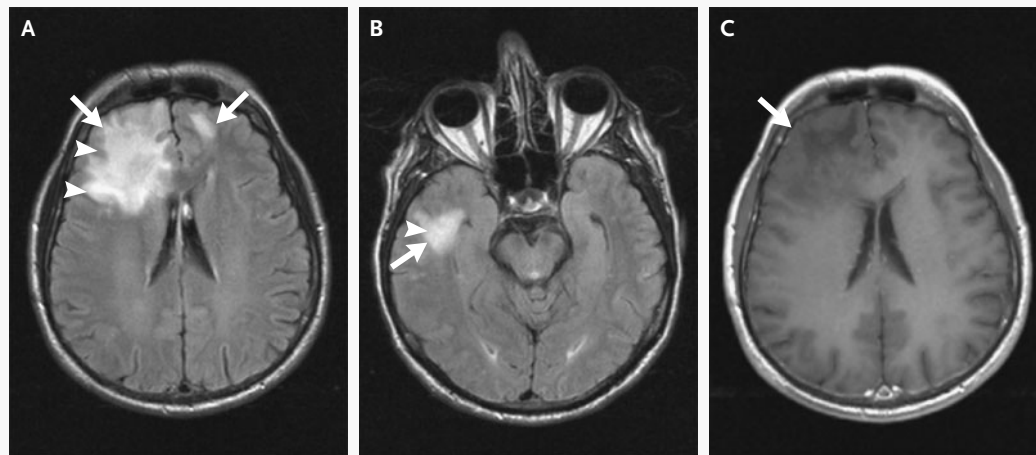


Figure 2. Initial MRI Findings.

In Panel A, a scan obtained with fluid-attenuated inversion recovery reveals a hyperintense lesion in the right frontal lobe and a smaller one in the left frontal region (arrows). A temporal lesion is indicated by the arrow in Panel B. These lesions were mainly confined to the white matter, as shown by the relative sparing of the cortex (arrowheads in Panels A and B), and were not enhanced by the administration of gadolinium on T_1 -weighted MRI (Panel C, arrow).

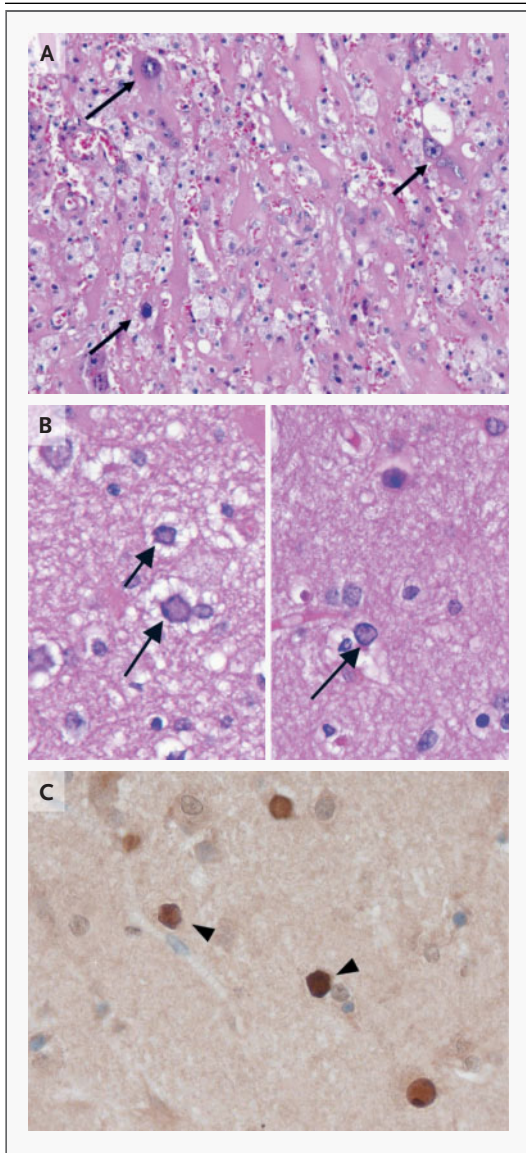


Figure 3. Histologic Findings.

Panel A shows enlarged astrocytes with atypical big nuclei (arrows) intermingled with foamy macrophages (hematoxylin and eosin). Panel B shows ground-glass inclusions of oligodendrocyte nuclei (arrows) (hematoxylin and eosin). Panel C shows rounded oligodendrocyte nuclei stained for polyomavirus antibodies (arrowheads). Immunoreactivity was mainly seen in cortical or subcortical oligodendrocyte nuclei. In the white matter, most of the tissue was destroyed, and staining was mainly seen in the nuclei of atypical astrocytes.

appearance and a basophilic rim of chromatin (Fig. 3B) in the relatively spared cortex, findings suggestive of PML. We then performed immunohistochemical analysis for polyomavirus proteins with mouse

monoclonal antibodies directed against the SV40 large T antigen (dilution, 1:10; Oncogene). We used an indirect immunoperoxidase technique (mouse Envision system, DakoCytomation) for detection. Immunohistochemical analysis revealed staining of atypical astrocyte nuclei as well as oligodendrocyte nuclei (Fig. 3C), a finding indicative of the presence of polyomavirus particles in the lesion and confirming the diagnosis of PML. In contrast to the positive staining in the brain lesion, the intestinal mucosa specimens obtained before the administration of natalizumab were uniformly negative.

DETECTION AND IDENTIFICATION OF JC VIRUS

To allow more specific detection of JC virus and to study the temporal relationship between the administration of natalizumab and viral replication, we analyzed the available serum samples and brain tissue for sequences of the JC virus genome. We performed a quantitative real-time polymerase-chain-reaction assay using primers specific for JC virus (PEP3 and PEP6), as described previously.⁶ No viral DNA was detected in serum obtained at multiple times from April 1999 through February 2003 (Table 1) or in the fresh-frozen intestinal samples obtained at surgery in 2000 while the patient was being treated with several potentially immunosuppressive drugs. However, JC virus DNA was detected in a serum sample obtained two months before admission, while the patient was receiving monthly infusions of 300 mg of natalizumab, and had increased by a factor of 12 by the time the patient was admitted (Table 1). In addition, the paraffin-embedded tissue of the brain lesion contained a high viral load (Table 1).

To identify the JC virus genotype, we amplified a 215-bp fragment of the VP1 major capsid protein, enabling us to differentiate among seven different genotypes and multiple subtypes.⁷ We sequenced the nucleotide fragments and looked for matches in the GenBank database (National Institutes of Health). JC virus genotype 2 was identified in both serum and brain tissue.

DISCUSSION

We report a fatal case of PML in a man with Crohn's disease treated with natalizumab. PML is a rare but often lethal and untreatable disorder of the central nervous system (CNS), with large white-matter lesions typically occurring in immunocompromised patients.^{8,9} The pandemic of the acquired immu-

Table 1. Time Course of JC Viral Load in Serum and Brain.

Tissue	Date	No. of Samples	Treatment*	Test for JC Virus DNA†
Serum	April–November 1999	6	Infliximab, azathioprine	Negative
	April 2000	1	Budesonide, azathioprine	Negative
	March–May 2002	6	Natalizumab, azathioprine	Negative
	June 2002–January 2003	10	Azathioprine (until November 2002)	Negative
	February 2003	1		Negative
	May 2003	1	300 mg of IV natalizumab per month (total, 3 doses), beginning in March 2002	520 copies/ml
	July 2003	1		6600 copies/ml
Brain	July 2003	1	300 mg of IV natalizumab per month (total, 5 doses), beginning in February 2003	500,000 copies/cell‡

* IV denotes intravenous.

† Serum samples containing fewer than 125 copies per milliliter were considered negative.

‡ The number shown is the median number of copies of JC virus DNA in DNA extracts from four slices (range, 2.2×10^5 to 3.9×10^6).

odeficiency syndrome has resulted in a sharp rise in deaths associated with PML, to an estimated 6.1 cases per 10 million persons in 1987,¹⁰ but PML also occurs in patients with impaired cellular immunity from other causes. In transplant recipients, the most frequently observed symptoms of PML are hemiparesis (in 50 percent), apathy (in 46 percent), and confusion (in 38 percent).¹¹ The pathogenesis of PML has been associated with reactivation of JC virus, a human polyomavirus. Polyomavirus infection is widespread, and antibodies are detected in serum in 50 to 85 percent of persons in the United States and Europe.¹² After primary infection, the virus resides in the kidney,¹³ and it is not entirely clear how the virus is transported to the brain. It is assumed that B cells deliver the virus to the oligodendrocytes, but active replication does not appear to occur in the blood.^{14,15}

Our patient had initially received a diagnosis of an astrocytoma on the basis of the predominant frontal lesion, a high number of atypical astrocyte nuclei, and an increase in the Ki67-MIB1 proliferation index in the resected frontal lesion. However, we revisited this patient's course after Elan and Biogen Idec had publicly released two case reports of PML in patients with multiple sclerosis treated with a combination of natalizumab and interferon beta-1a.⁴

In retrospect, initially circumstantial evidence was already present to support a diagnosis of PML, such as the absence of contrast enhancement in the brain lesions, the presence of multiple lesions,¹⁶ the presence of foamy macrophages, and the finding of

ground-glass nuclear oligodendrocyte inclusions on histologic examination.¹⁷ The diagnosis was not considered, however, because of the pathology report. Immunostaining for polyomaviral large T antigen provided strong evidence of the diagnosis of PML in this patient. Even more convincing were the findings of high levels of JC virus-specific DNA in the brain lesions and of the same JC virus genome sequences in the serum two months before the clinical onset of PML. We found the JC virus genotype 2 in serum and brain samples from our patient. JC virus genotypes 1 and 4 are predominant in Europe,¹⁸ but genotype 2 has been associated with the development of PML.^{18,19}

We identified a clear temporal relationship between the monthly natalizumab treatments and the occurrence of JC virus replication in our patient. Although he had been treated with corticosteroids and the immunomodulators infliximab (a monoclonal antibody against tumor necrosis factor) and azathioprine, JC virus DNA appeared in the serum only after the reintroduction of natalizumab as monotherapy. We do not know whether interrupting natalizumab therapy when the JC virus DNA first appeared in the serum would have prevented full-blown PML. An early analysis of cerebrospinal fluid for JC virus DNA would also have confirmed the occurrence of viral replication and is recommended when PML is clinically suspected.

Natalizumab, a humanized IgG4 antibody targeting α_4 integrins, is a member of an emerging class of drugs: the selective adhesion-molecule (SAM) inhibitors. The α_4 integrins are selectively

involved in leukocyte transport to the gut and the brain.²⁰ Natalizumab both blocks the engagement of $\alpha_4\beta_7$ integrin with endothelial mucosal addressin-cell adhesion molecule 1 in the gut and blocks the engagement of $\alpha_4\beta_1$ integrin with vascular-cell adhesion molecule 1, which is expressed on the endothelium of various organs, including the brain.²⁰ Two controlled trials suggesting the efficacy and tolerability of SAM inhibitors in patients with Crohn's disease^{2,3} led to two larger multicenter trials designed to evaluate the therapeutic potential of natalizumab: ENACT-1, involving the induction of clinical remission, and ENACT-2, involving the maintenance of natalizumab-induced remission. Our patient participated in both trials.

The ability of natalizumab to inhibit leukocyte transport to the gut and the CNS selectively has been invoked to explain the limited burden of infectious complications associated with the clinical use of this compound. Moreover, because α_4 integrins are not expressed by neutrophils, blocking the function of these receptors is unlikely to compromise the immune defense against bacterial infections. Nevertheless, the findings in our patient and in the two patients with multiple sclerosis demonstrate that the use of SAM inhibitors such as natalizumab can be associated with the reactivation of latent infection with JC virus. However, we cannot exclude the possibility that the intermittent lymphopenia in our

patient contributed to the reactivation of JC virus. JC virus replication could have started in the kidney or in lymphoid tissue, and natalizumab probably impaired JC virus–primed transport of CD4+ helper T cells and CD8+ cytotoxic T cells to the brain, resulting in fulminant viral replication in infected oligodendrocytes and astrocytes.

The respective roles of deficient CD4+ helper T cells and deficient CD8+ cytotoxic T cells in the reactivation of JC virus infection are still debated,²¹ but the inhibition of α_4 integrins has been shown to impede the transport of both types of cells to the CNS.²² Furthermore, $\alpha_4\beta_1$ integrin is also expressed at high levels on endothelial cells, which are an essential constituent of the blood–brain barrier.^{23,24} If β_1 integrins have a crucial role in stabilizing the blood–brain barrier, the inhibition of these molecules by natalizumab may also have facilitated the infiltration of JC virus particles into the CNS.

In conclusion, our case report demonstrates that polyomavirus replication leading to PML, a life-threatening disorder, can occur in patients who receive natalizumab. Since our patient had previously received other immunomodulatory agents with no reactivation of JC virus infection, further studies are needed to establish to what extent α_4 -integrin antibodies and other SAM inhibitors increase the risk of opportunistic CNS infection.

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