Fatal PML associated with efalizumab therapy

Insights into integrin $\alpha\text{L}\beta\text{2}$ in JC virus control

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ABSTRACT

Objectives: Progressive multifocal leukoencephalopathy (PML) has become much more common with monoclonal antibody treatment for multiple sclerosis and other immune-mediated disorders.

Methods: We report 2 patients with severe psoriasis and fatal PML treated for ≥ 3 years with efalizumab, a neutralizing antibody to $\alpha\text{L}\beta\text{2}$ -leukointegrin (LFA-1). In one patient, we conducted serial studies of peripheral blood and CSF including analyses of leukocyte phenotypes, migration ex vivo, and CDR3 spectratypes with controls coming from HIV-infected patients with PML. Extensive pathologic and histologic analysis was done on autopsy CNS tissue of both patients.

Results: Both patients developed progressive cognitive and motor deficits, and JC virus was identified in CSF. Despite treatment including plasma exchange (PE) and signs of immune reconstitution, both died of PML 2 and 6 months after disease onset. Neuropathologic examination confirmed PML. Efalizumab treatment was associated with reduced transendothelial migration by peripheral T cells in vitro. As expression levels of LFA-1 on peripheral T cells gradually rose after PE, in vitro migration increased. Peripheral and CSF T-cell spectratyping showed CD8+ T-cell clonal expansion but blunted activation, which was restored after PE.

Conclusions: From these data we propose that inhibition of peripheral and intrathecal T-cell activation and suppression of CNS effector-phase migration both characterize efalizumab-associated PML. LFA-1 may be a crucial factor in homeostatic JC virus control. *Neurology*®

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GLOSSARY

BBB = blood-brain barrier; **IRIS** = immune reconstitution inflammatory syndrome; **JCV** = JC virus; **PBMC** = peripheral blood mononuclear cell; **PE** = plasma exchange; **PML** = progressive multifocal leukoencephalopathy; **TCR** = T-cell receptor; **Tcm** = central memory T cells; **Tem** = effector memory T cells.

Progressive multifocal leukoencephalopathy (PML) is a serious demyelinating disease of the CNS occurring predominantly in immunocompromised patients (reviewed in Koráľnik¹) and is caused by JC virus (JCV), a 40-nm, nonenveloped, double-stranded DNA, icosahedral polyomavirus.² Serologic studies suggest prior JCV infection of 50%–80% of the general population.³ A neurotropic spread of replicating virus with development of PML occurs in diverse settings such as HIV infection, lymphoproliferative disorders, and organ transplantation.⁴ The risk of PML is increased by some immunomodulating therapies, including mycophenolate mofetil and monoclonal antibodies such as the anti-CD20 monoclonal antibody therapy rituximab.⁵ Particular interest has recently focused on the association of PML with

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Supplemental Data

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natalizumab,⁶ a humanized monoclonal antibody directed against α 4-integrin approved for treatment of relapsing forms of multiple sclerosis^{7,8} and Crohn disease.⁹

Efalizumab (Raptiva; Genentech, San Francisco, CA, and Merck-Serono, Geneva, Switzerland) is a humanized monoclonal antibody to the α -subunit of the integrin LFA-1 (CD11a) and was approved for the treatment of severe and therapy-refractory psoriasis until it was taken off the market in 2009 after the observation of PML. Efalizumab efficiently blocks CD11a on cells such as lymphocytes,¹⁰ thereby inhibiting their migration into the target tissue. In addition, efalizumab down-regulates CD11a on target cells.¹¹ In October and November 2008, 3 fatal cases of PML in patients treated with efalizumab were reported by the manufacturers.^{12,13} Here, we further describe 2 of those cases, including a detailed immunologic study of one case.

METHODS In both cases of PML, immunohistochemical analysis of brain tissue was performed to illustrate pathology, JCV infection, and immune cell infiltrates. For patient 1, we combined several methods for an immunologic workup: flow cytometric stainings of surface molecules and of bound efalizumab, transmigration assays over primary human endothelial cells, and T-cell receptor repertoire analysis via multiplex PCRs. A detailed description of the performed protocols can be found in e-Methods on the *Neurology*[®] Web site at www.neurology.org.

Standard protocol approvals, registrations, and patient consents. The local ethics committees in Würzburg and Cleveland approved the use of human subjects for this study. All patients gave informed written consent to participate in the study at a time when they were still competent to understand the usefulness of the study.

CASE REPORTS **Patient 1.** An HLA-A2+, 47-year-old man presented with progressive left hemiparesis, dysarthria, and memory difficulty. Past medical history was notable for psoriasis, for which he was treated sequentially with retinoic acid, PUVA therapy, fumaric acid, and methotrexate (November 2002–May 2003). He was then treated with efalizumab (1 mg/kg by weekly subcutaneous application) from 2006 on for 3 years before the diagnosis of PML was made. Efalizumab was discontinued 3 months after symptom onset. Brain MRI at that time showed multiple foci of increased T2 signal in the frontal and parietal lobes bilaterally. CSF showed 5 cells/ μ L, all mononuclear, with normal protein, negative oligoclonal bands, and detection of JCV by PCR (1,860 copies/mL), all confirming PML. Complete CSF data are found in figure 1.

Treatment for PML included 5 courses of plasma exchange (PE) (30 mL/kg body weight per exchange) over 11 days, to accelerate depletion of efalizumab¹⁴ and mefloquine (1,000 mg loading dose, then 250 mg PO weekly), based on preliminary reports of antiviral effects in vitro.¹⁵ Over the following several weeks, left hemiparesis progressed and left-sided neglect was observed. Repeat CSF studies showed normal cell count, but increased JCV load (20,500 copies/mL). In view of the relentlessly progressive neurologic disorder, a prolonged course of IV immunoglobulin (240 g total) was given with the rationale that it might downregulate a potentially fulminant immune reconstitution inflammatory syndrome (IRIS), although there is currently no direct evidence supporting this treatment. The clinical signs and course of IRIS observed in this patient appeared rather unusual and did not present as an acute or sub-acute neurologic deterioration. Still, the tentative diagnosis of IRIS was made in view of all clinical, imaging, and CSF analyses. The patient developed seizures and further declined mentally. MRI showed further progression of CNS lesions, but no contrast enhancement. CSF studies now demonstrated pleocytosis (138 cells/ μ L) and further increased JCV load (106,000 copies/mL), supporting the diagnosis of IRIS. Seizures were successfully treated with valproic acid. Over the next weeks, the patient became stuporous and died in coma 6 months after symptom onset. A complete autopsy was performed.

Patient 2. A 70-year-old man presented with a 1-week history of depression, headache, social with-

Figure 1

CSF laboratory findings in patient 1

Hospital day	3	21	30	42	58
	5x PE	IVIG			
Treatment		Mefloquine			
		Mirtazapine			
Leukocytes, μ L ⁻¹	5	3	138	28	14
JCV DNA, copies/mL	1,860	20,500	106,000	1,860	3,960
β_2 -MG, mg/L		2.0	2.8	3.2	4.4
IgG, mg/dL			8.9	5.4	7.3

Abbreviations: β_2 -MG = β_2 -microglobulin; IgG = immunoglobulin G; IV Ig = IV immunoglobulin; JCV = JC virus; PE = plasma exchange.

Figure 2 Neuropathologic investigation of autopsy CNS tissue from the 2 patients with progressive multifocal leukoencephalopathy

Representative examples of postmortem pathology are shown. The origin of the samples is indicated by patient number in parentheses. (A) Macroscopic image of the occipital lobe (2). The arrowheads show areas of softening and the arrow highlights an area with pronounced cavitation. (B) Scan of a Luxol fast blue (LFB)-hematoxylin & eosin (H&E)-stained section showing a large confluent area of demyelination (2). (C) Microscopic image showing patchy foci of demyelination (2). (D) Macrophages containing LFB-positive myelin debris (arrowheads) and an oligodendrocyte with an intranuclear viral inclusion (arrow) (2). (E) JC virus demonstrated by JC virus-specific immunoreactivity (arrowhead) (1). (F) Ultrastructural demonstration of JC virus in an oligodendrocyte nucleus (2). (G) Perivascular inflammatory infiltrate (H&E) (1). (H-K) Immunohistochemistry for CD3 (H), CD8 (I), CD4 (J), and CD20 (K) (1). (L, M) Plasma cells as demonstrated by H&E (L) and CD138 immunostaining (M) (1). Scale bar = 2 mm (B), 200 μ m (C), 20 μ m (D, E, and L), 50 μ m (H, I, J, K, and M), 100 μ m (G), and 50 nm (F).

drawal, language difficulties, and peculiar behavior. Past medical history was notable for psoriasis for 22 years, during the last 4 of which he received efalizumab (1 mg/kg subcutaneous weekly). Before treatment with efalizumab, the patient did not take any systemic medications for psoriasis and received only topical creams, including those containing coal tar solution, salicylic acid, and sulfasalazine. Brain MRI showed increased T2 signal in the bilateral cerebellar, left parietal, occipital, and right medial temporal lobes, and, in addition, a left posterior temporal venous angioma (figure e-1). Mental status declined over the next several weeks. Repeat brain MRI showed progression of

brain lesions, none of which enhanced. Results of routine CSF studies (which did not include JCV PCR) were unremarkable. Efalizumab was discontinued 1 month after symptom onset.

Mental status continued to decline over the next several weeks. He developed marked ataxia, visual tracking difficulty, and suicidal ideation. Repeat CSF testing showed JCV by PCR, and PML was diagnosed. He was treated with 3 courses of PE (1 plasma volume per exchange) and mefloquine (750 mg PO weekly).

Despite therapy, he developed right hemiparesis and became increasingly confused and agitated. Repeat brain MRI 10 days after PE showed extensive confluent

lesions with laminar necrosis in the lateral left parietal lobe and enhancement involving the medial left occipital-parietal lobe. He became obtunded and died 2 months after onset of symptoms. A brain-only autopsy was performed.

RESULTS Histopathology. The macroscopic and microscopic features of both brains were similar and diagnostic of PML. No other significant neuropathology was observed. Macroscopically, multiple regions of softening and cavitation were seen in the white matter of the cerebrum (figure 2A) and cerebellum. Microscopically, these lesions corresponded to myelin loss (figure 2, B and C) with relative sparing of axons at a variable degree (not shown). The demyelinated areas ranged from large and confluent lesions in regions of gross abnormality (figure 2B) to multiple small foci of demyelination in macroscopically normal-appearing areas (figure 2C). Demyelinated areas showed macrophages with Luxol fast blue inclusions, reactive astrocytes, and oligodendrocytes with intranuclear viral inclusions (figure 2D). Affected oligodendrocytes were immunoreactive with antibodies to JCV antigens (figure 2E),¹⁶ and electron microscopy demonstrated viral particles (figure 2F). Perivascular lymphocytes were conspicuous in many lesions (figure 2G). CD3+ T lymphocytes were both perivascular and intraparenchymal (figure 2H). The majority of CD3+ T cells were CD8+ (figure 2I). CD4+ T cells and CD20+ B cells were also present (figure 2, J and K). In some lesions, plasma cells were identified by morphology and CD138 immunostaining (figure 2, L and M).

Serial assessments of CD4+ and CD8+ T cells during PML. Serial peripheral blood and CSF analyses were performed in patient 1 (peripheral blood at 8 time points and CSF at 5 time points). Assessments included efalizumab binding, migration capacity, CD4+ and CD8+ T-cell spectratypes (which indicate clonal expansions), naive, memory, and effector phenotype, and JCV specificity. Data comprised effects on CD4+ and CD8+ T cells of PML during efalizumab treatment and the response to PE-mediated removal of efalizumab.

Efalizumab binding. Efalizumab binding to T cells diminished after PE and reached the level found in untreated subjects about 3 weeks after the final PE (figure 3A).

In vitro migration assay. LFA-1, the molecular target of efalizumab, is involved in T-cell migration. To mimic the influence of efalizumab on the passage of immune cells across the blood-brain barrier (BBB),¹⁷ we assessed the migration of peripheral blood mono-

nuclear cells (PBMCs) in an in vitro model of the human BBB.¹⁸ On admission (e.g., before PE and in the presence of efalizumab), PBMCs migrated poorly. The migratory capacity began to increase 2 weeks after the initial PE and increased steadily thereafter (figure 3B). In line with the notion that efalizumab not only blocks LFA-1 but also induces downregulation of LFA-1 expression,¹⁹ T cells showed steadily increasing levels of surface LFA-1 after PE (figure 3C). Moreover, migrated CD8+ T cells showed a higher expression of LFA-1 than their nonmigrated counterparts (figure 3C), consistent with a crucial role of LFA-1 in this migration assay. As disease controls, we evaluated peripheral blood cells from patients with HIV-associated PML (n = 2) in this assay. The migratory rate of cells from patients with HIV-associated PML was similar to that of cells from healthy donors (n = 3), excluding the possibility that PML itself is associated with diminished migration. After PE, migration of cells obtained from the patients with efalizumab-associated PML was still impaired compared with that of controls (figure 3D).

CDR3 spectratyping of CSF cells. Using CDR3 spectratyping, we assessed the T-cell receptor (TCR) repertoire at several time points in peripheral blood and CSF during life and in different CNS regions at autopsy in patient 1. Spectratyping allows detection of clonal T-cell expansions, indicating a T-cell-mediated immune response.²⁰ Before PE, cells in the CSF showed few clonal expansions, indicating that the extent of CNS antigen-specific responses was limited. After PE, the numbers of clonally expanded cells rose, although the absolute cell numbers remained low. The presence of clonally expanded T cells in the CSF thus hints toward an early reconstitution of T-cell function (figure 3E). Subsequent pleocytosis was temporally associated with the rise of Gaussian-distributed TCRs (figure 1), putatively indicating restored T-cell migration into the CSF and intrathecal antigen-mediated restimulation.²¹ This pleocytosis coincided with the onset of IRIS (figure 3F, blue arrow). All CSF samples analyzed had a red blood cell count of <1/ μ L.

Naive, memory, and effector T cells. After PE, we used flow cytometry to assess naive, central memory (T_{cm}), and effector memory (T_{em}) bulk CD4+ and CD8+ T cells and other immune cell populations, both in the periphery and in the CNS^{22,23} (figure 3G, table e-1). Immediately after completing PE, there was a predominance of naive (CCR7+CD45RA+) CD4+ and CD8+ T cells in peripheral blood (47% and 54%, respectively), similarly to those observed before PE (table e-1) and in HIV-positive patients

Figure 3 Immunologic features of patient 1 during progressive multifocal leukoencephalopathy (PML)

(A-F) x-axis shows days in hospital, and time of plasma exchange (PE) treatment is indicated. (A) Binding of efalizumab to CD4+ and CD8+ T cells. MFI = mean fluorescence intensity. (B) T-cell migration over an in vitro blood-brain barrier. (C) LFA-1 expression on CD8+ T cells. GeoMean = geometric mean. (D) Number of migrated cells 2 and 6 weeks after PE. HIV-associated PML (PML (HIV); n = 2), healthy donors (HD; n = 2). (E) T-cell receptor repertoire of CSF cells. GD = Gaussian distributions. (F) Total cell count in the CSF. (G) Phenotypic analysis of T-cell subsets (naïve [Tnaïve], central memory [Tcm], and effector memory [Tem]) as analyzed by the staining for CD4, CD8, CD45RA, and CCR7. T-cell compartments of CD4+ (left row) and CD8+ T cells (right row) are shown for different time points and in different compartments (CSF). Shown are plots of CCR7 (x-axis) and CD45RA (y-axis). Naïve T cells are CCR7+CD45RA+, central memory T cells are CCR7+CD45RA-, and effector memory T cells are CCR7-CD45RA-. Distributions for CD4 and CD8 T cells are shown from CSF (hospital day 42, CSF). Representative examples of patients with HIV-associated PML (PML (HIV)) and healthy donors (HD) are shown. Ef = efalizumab. (H) JC virus (JCV) VP1_{p36}-specific CD8+ T cells in patient 1: peripheral blood mononuclear cell (PBMC) samples obtained before PE and 1 week after the end of the PE were cultured as described in Methods, and staining with HLA-A*0201/JCV VP1_{p36} tetramer was performed at day 14. Numbers in the plots represent percentage of cytokine-secreting CD8+ T cells. (I) Cytokine secretion of CD8+ T cells after stimulation with JCV VP1_{p36} in patient 1. PBMC samples obtained before PE (left row, hospital day 10) and 1 week after the end of the PE (right row, hospital day 22) were cultured as described in Methods and an intracellular cytokine staining consensus statement with HLA-A*0201/JCV VP1_{p36} peptide was performed at day 14. Numbers in the plots represent percentage of cytokine-secreting CD8+ T cells. IFN = interferon; SEB = staphylococcal enterotoxin B; TNF = tumor necrosis factor.

with PML, as well as in healthy donors. Within 3 weeks after the last PE, however, peripheral CD4+ T cells in the blood had shifted to be mostly Tcm (68%), whereas CD8+ T cells were mainly Tem (53%). At this time point, CSF showed mild pleocytosis (14 cells/mL) with 78% of CD8+ cells and 43% of CD4+ T cells being Tem, strikingly different from the virtual absence of Tem from CSF of healthy subjects or patients with multiple sclerosis. CD4+ Tem in the periphery did not reach high

numbers until the last available time point (5 weeks after PE). Of note, we did not observe an increase in the percentage of CD4+CD8+ double-positive T cells during or after PE in the periphery or in the CSF (data not shown).

JCV-specific CD8+ T cells. We assessed JCV-specific CD8+ T cells at 2 time points during the course of the disease: before PE (day 10 in the hospital) and after PE (day 22 in the hospital) (figure 3, H and I).

Figure 4 MRI scans and schematic distribution of CNS tissue samples of patient 1 with corresponding virus load and presence of clonally expanded T cells

(A) Initial MRI scan of patient 1 (hospital day 1, left panels) and last MRI scan on day 73 (right panels). (B) Schematic drawing of the sites of assessed samples from autopsy brain tissue. Depicted are findings from the 2 cut brain slices analyzed sagittally (frontal and parietal, each left and right hemisphere, left panel), the distribution of the samples (numbering) in the brain slices (middle panel; samples are always in the sequence of cortex [top], white matter [top], white matter [basal], cortex [basal]). The samples are correlated with the number of dominant clonal expansions in each sample (S), in the white matter of this hemispheric brain slice (WM), the gray matter (GM), the complete hemispheric brain slice (HS), the complete brain slice (BS), and the brain hemispheres (BH). The gray matter/cortex is always highlighted with gray, the right hemisphere with red, and the left hemisphere with green. The virus load in these samples is given in copies (C) per mL. In addition, kidney (K) was analyzed.

Using the HLA-A*0201/JCV VP1_{p36} peptide to stimulate total PBMCs for 14 days in vitro, we did not find any evidence of JCV-specific memory CD8⁺ T cells either before or after the PE. There was no expansion of JCV tetramer-specific CD8⁺ T cells or secretion of inflammatory cytokines (interferon- γ and tumor necrosis factor- α) by these cells. In line with these data, spectratyping of T cells in CNS, in CSF, and in the periphery did show various clonal expansions, none of which could be attributed to JCV specificity (figure e-2A). However, there were clonal expansions detected in more than one tissue compartment. One notable V β 6⁺ CD8⁺ clone (V β 6 CASS LGAT NEKLF J β 1.4) was prominently expanded in CSF,

CNS, and peripheral blood at more than one time point. We assume that this clone is myelin-specific as suggested by another study.²⁴ This notion is in line with our data showing massive, putatively non-JCV-related T-cell reactions in periphery and CSF.

V β expansions in the T cells from CNS autopsy tissue. TCR repertoire and viral load were evaluated in various regions. The MRI scans taken at 2 time points are shown to illustrate the sites of progressive CNS pathology over a long time period (figure 4A). CNS tissues taken from the regions indicated in figure 4B showed various degrees of clonal expansions (figure e-2B), with white matter

containing larger numbers of expansions than gray matter (figure 4B).

DISCUSSION We present 2 fatal cases of efalizumab-associated PML, both after a similar course of disease. In patient 1, we performed serial T-cell phenotyping and functional assessments of T cells from blood, CSF, and brain tissue.

Three months after onset of clinical PML, the majority of peripheral T cells in patient 1 showed a naive phenotype consistent with lack of differentiation toward effector functions, which was in line with the lack of clonal expansions in CSF and periphery. As expected, blockade of LFA-1 also dramatically impaired T-cell migration.

Removal of efalizumab by PE induced a series of immunologic changes. Immediately after PE, clonal expansions in the CSF indicated the reconstitution of the proliferative function of primed central T cells. The subsequent appearance of effector memory lymphocytes (CD8⁺ T cells 3 weeks after PE and CD4⁺ T cells 5 weeks after PE) indicates a strong peripheral immune reaction with the potential for enhanced T-cell migration across the blood-CSF barrier.²⁵ The absence of a virus-specific immune response before and after PE was documented by lack of JCV-specific memory CD8⁺ T cells and was observed despite several months of clinically evident PML.²⁶ We propose that the increase in clonal CD8⁺ T-cell numbers seen after PE in patient 1 represented a poly-specific expansion of CD8⁺ T cells rather than of JCV-specific CD8⁺ T cells. This hypothesis is consistent with the observed increase in CSF viral load after PE, plausibly related both to insufficient virus control and BBB malfunction along with incipient IRIS. This time point of the disease course (hospital day 22) also corresponded to the restoration of memory CD8⁺ T-cell activation and accumulation of CD8⁺ T cells in the CSF, a site of immune surveillance where T cells are restimulated by local antigen presentation.^{17,27–30} The restimulation of lymphocytes in the subarachnoid space requires physical interactions between T-cell LFA-1 and antigen-presenting cell (for CD4⁺ T cells) or target cell (for CD8⁺ T cells) intercellular adhesion molecule-1.³¹ Lymphocyte restimulation by antigen elicits production of cytokines, thereby influencing the cerebrovascular endothelium and promoting transmigration of leukocytes across the BBB.²⁵ Thus, efalizumab seems to impair 3 aspects of adaptive antiviral immunity: 1) activation and proliferation of naive T cells; 2) restimulation of memory T cells

with antigen; and 3) T-cell migration to sites of infection.¹⁷ In agreement with the hypothesis that T-cell surveillance is crucial to hold JCV in check, the migration of T cells was indeed impaired: in vitro BBB assays showed that cells from patient 1 migrated poorly before PE and slowly regained migratory function after efalizumab removal, but never reaching healthy control levels. Improvement of migration was observed in vitro after about 50% of cell-associated efalizumab was removed, corresponding to recovery of LFA-1 expression on lymphocytes.

The central phase of immune reconstitution was followed by recovery of peripheral immune cell function, including T-cell migration and maturation. Subsequent parenchymal inflammation associated with IRIS was reflected by increasing lesions on MRI without contrast enhancement and clinically by newly occurring seizures along with deterioration of vigilance.

Features of this patient's immunologic evaluation suggest that the mechanisms by which efalizumab and natalizumab lead to PML are different. First, efalizumab does not cause release of CD34⁺ bone marrow progenitor cells into the circulation (table e-1), a prominent effect of natalizumab, which could possibly lead to release of virus from a bone marrow site of nonpathogenic replication.³² Moreover, natalizumab, but not efalizumab, reduces CSF cell counts by more than 70%.³³ Because T-cell immune surveillance of the CNS takes place in the CSF-containing subarachnoid space,³⁴ natalizumab limits cells available to perform this function. In contrast, efalizumab appears to impair intrathecal antigen-presenting cell/antigen-mediated restimulation of CD4⁺ and for CD8⁺ T cells. Both natalizumab and efalizumab block and downregulate leukocyte-derived integrins, through which T cells cross the BBB to eliminate pathogens and virus-infected cells, and both monoclonal antibodies have similar pharmacokinetics.^{35,36} Importantly in patient 1, most of the clonally expanded T cells were found in the CNS. Only occasionally were individual expanded T-cell clones found in blood, CSF, and CNS tissue because the blockade of LFA-1 prevented migration of effector cells across the BBB. Clonal expansions in the CNS were numerous, indicating that the immune activation and T-cell expansion in patient 1 probably occurred within the parenchyma. Of note, in comparison with natalizumab, for which roughly 75% of patients with PML survive,³⁷ all 3 patients with efalizumab-related PML died. From our data,

it can be speculated that interference with LFA-1 might be associated with several steps of JCV defense including immune cell migration, T-cell priming and subsequent restimulation, and target cell lysis.

Our data allow formulation of a provisional hypothesis relating to JCV immune surveillance under physiologic conditions, its failure due to efalizumab, and the immune reconstitution after PE (figure e-3). We suggest that efalizumab inhibits antiviral T-cell activation and expansion as well as migration across the BBB, intrathecal lymphocyte restimulation with antigen, and target cell lysis, leading to development of PML. The cases described in this report may help

to conceive strategies to interfere with the malfunction of immune cells and of the BBB that are relevant for the prevention and effective treatment of PML.

AUTHOR CONTRIBUTIONS

Dr. Schwab performed experiments, analyzed the data, and wrote the first draft of the manuscript. Dr. Ulzheimer coordinated patient recruitment and participated in writing the first draft of the manuscript. Dr. Fox coordinated experiments and edited the manuscript. T. Schneider-Hohendorf performed experiments and analyzed the data. Dr. Kieseier coordinated patient recruitment and edited the manuscript. Dr. C. Monoranu coordinated sample processing and analyzed neuropathology sections. Dr. Staugaitis performed histologic analyses, coordinated sample processing, and edited the manuscript. Dr. Welch coordinated patient recruitment and sample logistics. Dr. Jilek performed experiments and analyzed the JCV immunology data. Dr. Du Pasquier analyzed the JCV immunology data and edited the manuscript. Dr. Brück analyzed neuropathology sections and edited the manuscript. Dr. Toyka coordinated patient care and recruitment and edited the manuscript. Dr. Ransohoff coordinated experiments and data analyses, and participated in writing and editing the manuscript. Dr. Wiendl coordinated experiments, data processing, and participated in writing and editing the manuscript.

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DISCLOSURE

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Comment: PML and adhesion molecule therapy

A total of 159 cases of progressive multifocal leukoencephalopathy (PML) have now been associated with natalizumab, an antibody that inhibits CNS lymphocyte entry by binding to $\alpha 4$ integrin.¹ This study describes 2 of the 3 known fatal PML cases occurring following treatment with efalizumab, an adhesion molecule inhibitor that binds to the α subunit of LFA-1 (CD11a) and blocks its attachment to ICAM-1.² The estimated risk of PML in patients treated with efalizumab (~1:400) falls into the general range of that for natalizumab (~1:600), and resulted in the removal of efalizumab from the market in 2009. A unique feature of this study was the opportunity, in a single patient, to perform serial T-cell phenotyping and functional studies on blood and CSF. Efalizumab downregulated LFA-1 expression on CD8+ T cells and inhibited peripheral blood mononuclear cell (PBMC) migration in an in vitro model of the blood-brain barrier. Despite documentation of a clonal intrathecal T-cell response, the patient had no significant detectable JCV-specific CD8+ T-cell responses against a peptide fragment of the VP1 major outer capsid protein, a feature associated with disease progression and poor prognosis in other settings. Nonetheless, the patient developed immune reconstitution inflammatory syndrome (IRIS), suggesting that this assay only incompletely captures the spectrum of JCV-specific T-cell responses resulting in IRIS.³ Efalizumab removal resulted in reduced PBMC LFA-1 blockade, enhanced CD8+ T-cell migration capacity, and improved T-cell proliferative function. Although limited to a single patient, this study adds an important piece to the puzzle of PML associated with biological immunomodulatory therapies. Although the authors suggest that natalizumab and efalizumab differ in the mechanism by which they lead to PML, a far more parsimonious interpretation is that they are in fact quite similar in their mutual capacity to impair CNS T-cell migration and the associated antiviral surveillance functions that are likely critical to hold JCV in check.

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Disclosure: Dr. Tyler serves on scientific advisory boards for Pfizer Inc, Macrogenics, and PML Consortium; serves as an Associate Editor for the *Journal of Neurovirology* and *Neurology Today* and on the editorial boards of *Annals of Neurology*, *Apoptosis*, *Archives of Neurology*, *Experimental Neurology*, *the Journal of Infectious Disease*, *Microbial Pathogenesis*, and *Virology*; receives publishing royalties for *Handbook of Clinical Neurology*, *History of Neurology*, vol. 95 (Elsevier, 2010), and *Harrison's Principles & Practices of Internal Medicine*, 18th edition (McGraw-Hill, 2011); serves as a consultant for Aegis Creative Communication, Biogen Idec, Genentech, Inc., Janssen, Johnson & Johnson, and Roche; receives research support from the NIH and the US Department of Veterans Affairs; and has reviewed files and provided depositional testimony in medical-legal cases. Dr. Bennett has received speaker honoraria from Teva Pharmaceutical Industries Ltd.; has a patent pending re: Novel monoclonal antibody therapy for neuromyelitis optica; serves as a consultant for EMD Serono, Inc., Biogen Idec, and Pharmaceutical Industries Ltd.; and receives research support from EMD Serono, Inc., the National Multiple Sclerosis Society, and the Guthy-Jackson Foundation.

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