Treatment of Progressive Multifocal Leukoencephalopathy With Interleukin 7

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Progressive multifocal leukoencephalopathy (PML) is a demyelinating disorder of the brain caused by JC virus (JCV) replication in oligodendrocytes. The disease affects immunocompromised individuals and is usually fatal within months. Delgado-Alvarado et al described 1 patient with PML and idiopathic CD4+ T-cell lymphocytopenia (ICL) and reviewed 12 previous cases. Idiopathic CD4+ T-cell lymphocytopenia is diagnosed when absolute CD4+ T-cell counts are less than 300/μL on 2 or more occasions in the absence of potential causes of immunodeficiency, including immunosuppressive therapies and infections with human immunodeficiency virus (HIV) and human T-lymphotropic virus. We diagnosed ICL and PML in a patient and treated him with recombinant human interleukin 7 (rhIL-7).

Report of a Case

On May 16, 2012, a 61-year-old man was treated for an assumed stroke in his left cerebral hemisphere. The National Institutes of Health Stroke Scale (NIHSS) score was 2 (Figure 1A), and brain magnetic resonance imaging (MRI) revealed patchy high-signal lesions in the left middle cerebral artery territory (Figure 1B). Because of progressive weakness, aphasia, and neurocognitive impairment, the patient was referred to our clinic for a second opinion.

At presentation on August 18, 2012, physical examination revealed expressive aphasia, a right-sided spastic hemiparesis, dysarthria, and left parietal lobe dysfunction. To quantify the impairment, the NIHSS was expanded by 5 items (eTable in the Supplement), and the adapted National Institutes of Health Stroke Scale (aNIHSS) score was 10.4 Subsequent MRI revealed a T2-weighted, large, left subcortical hyperintense lesion (Figure 1B) and a T1-weighted marked hypointense lesion without contrast enhancement or mass effect. The result of a highly sensitive quantitative polymerase chain reaction (PCR) for JCV DNA in the cerebrospinal fluid (CSF) was negative. Laboratory test results were normal, but peripheral blood flow cytometry revealed low CD4+ T-cell counts (110/μL). Subsequent CD4+ T-cell counts

IMPORTANCE No reliable treatment options are known for progressive multifocal leukoencephalopathy with underlying immunodeficiency. We describe successful compassionate use of recombinant human interleukin 7 (rhIL-7).

OBSERVATIONS After the diagnoses of progressive multifocal leukoencephalopathy and idiopathic CD4+ T-cell lymphocytopenia were established, a 61-year-old man was treated with recombinant human interleukin 7 on November 1, 2012. Except for an episode of epilepsy partialis continua on January 16, 2013, a gradual clinical improvement was observed until March. Abnormalities shown on magnetic resonance imaging regressed; JC virus DNA in plasma, likely originating from the brain based on sequencing data, cleared; and increases in peripheral CD4+ T cells and JC virus intrathecal antibodies were observed. One year after treatment, the CD4+ T-cell count returned to baseline and the clinical improvement waned, possibly due to the patient’s complex epilepsy. On the latest evaluation on January 14, 2014, the patient’s condition was unchanged, with no signs of ongoing central nervous system infection.

CONCLUSIONS AND RELEVANCE The present case argues strongly for proof of the treatment concept. However, deeper insight into the JC virus and its pathogenesis and the immune response during central nervous system infection as well as further clinical studies are needed before recombinant human interleukin 7 can be recommended for the treatment of other cases of immunodeficiency and progressive multifocal leukoencephalopathy.

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confirmed a diagnosis of ICL. The results of extensive malignancy screening were negative. Serologic test results were negative for HIV, human T-lymphotropic virus, herpes simplex virus, varicella zoster virus, hepatitis B virus, hepatitis C virus, Toxoplasmosis gondii, and Treponema pallidum, as were PCR analyses of HIV, Epstein-Barr virus, and cytomegalovirus. A stereotactic brain biopsy was performed in September, and histologic analysis, including immunohistochemical analysis, revealed characteristic findings of PML (Figure 1C). Quantitative PCR on DNA from a fresh brain biopsy performed on September 6, 2012, confirmed a diagnosis of PML. Three subsequent plasma analyses revealed low levels of JC virus (JCV) DNA. The first dose of recombinant human interleukin 7 (rhIL-7; CYT107, 10 μg/kg) was given on November 1, 2012. There was a significant increase in CD4+ and CD8+ T-cell counts at day 32. A temporary decrease in the natural killer (NK) cell count was observed during the third week of treatment. A marked clinical improvement was observed until January 16, 2013, when the patient was hospitalized due to epilepsy partialis continua. B, T2-weighted image taken at first symptoms of PML reveals multiple hyperintense subcortical lesions in the left hemisphere (left; May 15, 2012). Four months later, there is a confluent and large lesion in the same area (middle; October 1, 2012). Six months after treatment with rhIL-7 (CYT107) and 1 year after the first signs of the disease, there is regression of the pathologic signals (right; April 10, 2013). C, Hematoxylin-eosin staining (original magnification ×40) of the stereotactic brain biopsy specimen reveals reactive astrocytes, macrophages, perivascular inflammation, and a few enlarged oligodendroglial nuclei with dark violet viral inclusions (arrowhead). The insert (original magnification ×60) reveals immunohistochemical detection of JCV capsid protein in the nucleus of an infected oligodendrocyte (arrowhead) and the cytoplasm of astrocytes. A cross-reaction polyclonal rabbit serum directed against the capsid protein VP1 of the closely related BK virus was used. Of note, no BK virus DNA was detected by BK virus quantitative polymerase chain reaction. aNIHSS indicates adapted National Institutes of Health Stroke Scale.
biopsy specimen revealed $10^5$ JCV genomic equivalents per microgram of tissue. During the 2 months after the diagnosis of PML, low levels of JCV DNA (37, 60, and 78 genomic equivalents per milliliter) were detected in plasma. Despite additional PCR analyses, JCV DNA was not detected in CSF, urine, bone marrow, or peripheral blood mononuclear cells (PBMCs).

Treatment with mirtazapine, previously suggested to inhibit JCV replication, was started; however, the patient's condition worsened. Six months after the onset of PML symptoms, the aNIHSS score was 13. Mirtazapine treatment was discontinued, and on November 1, 2012, rhIL-7 (CYT107, 10 µg/kg) was administered subcutaneously once weekly for 3 consecutive weeks. Two and a half months after the first dose of rhIL-7 (on January 17, 2013), the patient was treated with a combination of levetiracetam, valproate sodium, and lacosamide for epilepsia partialis continua (Video). After 2 days the seizures disappeared, but residual twitching (clonic jerks) during use of the right arm continued to occur, sometimes interspersed with right-sided focal tonic-clonic seizures. A small dose of clonazepam was later added to his antiepileptic treatment. Brain MRI revealed regression of the pathologic signals but a slight increase in subcortical atrophy (Figure 1B). One year after the initial presentation, the patient had a Karnofsky performance status score of 60 and an aNIHSS score of 10. One year after treatment, the aNIHSS score had increased to 12, and MRI revealed further atrophy (not shown), but there were no signs of PML relapse and no viral IgM, followed by regression of MRI pathologic findings (Figure 1A). No additional immune responses were observed. On the basis of the long-term data, rhIL-7 was tolerated without any adverse effects. Clinical improvement was seen after 4 weeks, paralleled by increases in CD4+ T cells from approximately 130/µL to 200/µL. Moreover, viral clearance from plasma, increasing intrathecal JCV IgG and serum IgM, followed by regression of MRI pathologic findings were observed. On the basis of the long-term data, rhIL-7 caused a temporary improvement in the CD4+ T-cell counts, which seemed sufficient to battle the JCV brain infection. On the basis of a single case, conclusions should be drawn with caution, but several arguments support a potential favorable effect of rhIL-7.

**Leukocyte Levels After rhIL-7 Treatment**

The CD4+ and CD8+ T-cell counts were measured before each rhIL-7 administration and intermittently thereafter (Figure 1A). Three subsequent CD4+ and CD8+ counts (September 12, October 10, and November 1, 2012) were 100, 110, and 130/µL and 280, 220, and 280/µL, respectively. On day 32 after treatment, the CD4+ cell count significantly increased to 220/µL (paired samples t test, $df = 2, P = .01$) and the CD8+ cell count to 330/µL ($P = .04$). Notably, the increase in T-cell counts was followed by a temporary reduction of natural killer cells (Figure 1A). One year after rhIL-7 treatment, the CD4+ T-cell count was 150/µL.

**JCV DNA Sequences in Brain and Plasma**

The JCV genomes from the central nervous system (CNS) of patients with PML are characterized by the rearranged noncoding control region (rr-NCCR) due to partial deletions and duplications. Moreover, the JCV VP1 gene (GenBank KJ659288) encoding the protein responsible for host cell receptor binding is frequently mutated in patients with PML. To investigate this, long-range PCR was used to amplify the complete JCV genome from the brain biopsy specimen followed by cloning and whole-genome sequencing. The NCCR and VP1 region in plasma were separately amplified, cloned, and sequenced. The sequences revealed that the JCV belonged to genotype 1B, a common genotype in Europe. Five unique but closely related rr-NCCRs, with a signature rearrangement that consisted of a short D-block fragment, were found in the brain biopsy specimen and denoted University Hospital of North Norway patient 2 CNS strains 1 through 5 (Figure 2A). In plasma, only 1 closely related rr-NCCR variant (University Hospital of North Norway patient 2 plasma strain 1) was found.

Sequencing of the brain-derived VP1 gene revealed few changes compared with a reference strain CY (Figure 2B), but some genomes had a mutation, resulting in amino acid substitutions N265D or D66G, previously reported to be associated with PML. The VP1 sequences from plasma were almost identical to the brain sequences except for additional mutations in amino acids 62 and 55. The latter, L55F, is known to be a PML-associated mutation (Figure 2B).

**Immune Response**

The JCV-specific IgG antibody titers were strongly positive in CSF and serum at the time of the first rhIL-7 administration and 1 month later. According to the Reiber coefficient (Q), the blood CSF barrier was in the age-corrected reference range (Qalbumin, 8.2; QIgG, 4.4) but became slightly impaired at the second time point (Qalbumin, 9.1; QIgG, 3.7). The JCV-specific antibody index (QJCV/QIgG) was highly positive at 6.8 and 10.3 after rhIL-7 (reference <1.5), respectively. In serum, JCV-specific IgM titers increased from 1:400 to 1:3200 after administration of rhIL-7 (Figure 3A). Testing of JCV-specific T cells by interferon-γ enzyme-linked immunosorbent spot assays revealed an increase in spot-forming units first for capsid VP1 peptide epitopes and then for large T-antigen peptide epitopes in PBMCs after rhIL-7 administration (Figure 3B).

**Discussion**

Effective treatments for PML are lacking, and standard therapies to increase CD4+ T cells and/or improve immune function in ICL do not exist. In our patient, treatment with rhIL-7 was tolerated without any adverse effects. Clinical improvement was seen after 4 weeks, paralleled by increases in CD4+ T cells from approximately 130/µL to 200/µL. Moreover, viral clearance from plasma, increasing intrathecal JCV IgG and serum IgM, followed by regression of MRI pathologic findings were observed. On the basis of the long-term data, rhIL-7 caused a temporary improvement in the CD4+ T-cell counts, which seemed sufficient to battle the JCV brain infection. On the basis of a single case, conclusions should be drawn with caution, but several arguments support a potential favorable effect of rhIL-7.
Studies in HIV-positive patients indicate that increases of CD4⁺ T-cell counts from 100/μL to 200/μL are associated with decreased morbidity and mortality¹¹ and that CD4⁺ T-cell counts greater than 200/μL are associated with improved PML outcomes.²³ Although CD8⁺ cytotoxic T cells represent the key immune effectors controlling JCV replication, data indicate that JCV-specific CD4⁺ T cells are important markers of immune protection.²⁴ One of the key drivers of T-cell homeostasis and function is IL-7, and preclinical data indicate that this cytokine may have therapeutic effects in several clinical settings, including ICL.²⁵

Intrathecal antibody production may be an adjunct diagnostic tool in patients without detectable JCV DNA in CSF.²⁶ Although JCV-specific antibodies cannot protect patients from PML, increases in antibody titers may be a marker of immune recovery.²⁷ In other viral CNS infections, such as measles, CD4⁺ T cells have a critical role in augmenting B-cell antibody and CD8⁺ T-cell responses, and the presence of JCV-specific CD8⁺ T cells is linked to recovery from PML. Thus, the present case suggests that rHL-7 not only caused a rapid increase in overall CD4⁺ T-cell counts but also was associated with functional and clinical benefits.

The plasma initially tested had a low positive result for JCV DNA, but the results became negative after the patient started rhIL-7 treatment. The significance of this is unclear because the plasma JCV load has not been accepted as a marker of PML disease. In contrast, rearrangements of the NCCR have been associated with PML. In our patient, 6 related but different rr-NCCRs were found in the brain and plasma, and all lacked almost the entire D-block, which has been reported to strongly increase early gene expression and viral replication in vitro. Of note, the least complex rr-NCCR found may have been the precursor for all other NCCR variants found.

It is unclear how JCV reaches the CNS. Bone marrow–derived lymphocytes and B cells may be involved in transport into the CNS.³ In several attempts, we were unable to detect JCV DNA in PBMCs of our patient. Although the origin of the plasma variant is unknown, the complex plasma rr-NCCR, which was clearly related to the rr-NCCRs in the brain, suggests it resulted from leakage of a highly active, well-perfused PML lesion. This hypothesis is also supported by our finding of the PML-associated L55F mutation, which is thought to reduce receptor binding⁶ only in plasma. Our results also suggest that NCCR rearrangements occur independent of and, in our case, before VP1 mutations in PML.
Figure 3. JC Virus (JCV)-Specific Immune Responses

A, JCV-specific antibody response at the start and 4 weeks after recombinant human interleukin 7 (rhIL-7) treatment. The titers were determined by serial dilution using an enzyme-linked immunosorbent assay as reported10 with the corresponding cutoff at an optical density of 405 nm (OD_{405}) of 0.100. JCV-specific IgM titers increased from 1:400 to 1:3200 after administration of rhIL-7. B, JCV-specific T-cell responses by interferon-γ enzyme-linked immunosorbent spot assay. Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed, washed in RPMI culture medium that contained 5% human serum, and incubated overnight in culture medium in a 6-well plate.

After 24 hours, activated monocytes were pulsed with a pool of 15mer peptides (15m OPP) overlapping the entire viral capsid VP1 sequence (VP1 15m OPP; light blue bars) or the entire viral large T-antigen sequence (LTag 15m OPP; dark blue bars) (1 μg/mL). Lymphocytes were added and tested after 1 week by interferon-γ enzyme-linked immunosorbent spot assay after restimulation with the same peptide pools. Error bars indicate SD.

*IgG titer.

*a IgM titer.
Conclusions

In the absence of antiviral drugs, immune recovery has been the only hope for PML survival. The present case indicates that rhIL-7 treatment has the potential to improve CD4+ T-cell counts in ICL, with immunologic benefits that are crucial to the management of PML. Deeper insight into JCV pathogenesis and the immune response during CNS infection and further clinical studies are needed before rhIL-7 can be recommended for other cases of immunodeficiency and PML.

REFERENCES