Epstein-Barr virus-associated primary central nervous system lymphoma in an immunosuppressed patient with a comorbid autoimmune disorder: A case report

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Abstract. Patients with primary central nervous system lymphoma (PCNSL) typically present with non-focal neurological symptoms, including disorientation, poor balance and memory loss with unifocal or multifocal periventricular lesions seen on MRI. Deviations from these characteristic findings can delay diagnosis and lead to additional diagnostic tests being needed. The present study reports a 68-year-old man with a recent varicella zoster infection and history of acetylcholine receptor antibody-positive myasthenia gravis who received mycophenolate mofetil for 22 years. He presented with left eye vision changes and cognitive memory deficits. A brain MRI showed an enhancing lesion within his left medulla extending to the cerebellum. Cerebrospinal fluid analysis was positive for Epstein-Barr virus (EBV) and negative for malignancy. He was diagnosed with varicella zoster virus vasculopathy. At 3 months later, a repeat brain MRI showed multiple new enhancing lesions developing bilaterally along the periventricular white matter. Soon after, he presented to a local ER with acute left-sided blurry vision and worsening memory loss, and he began receiving steroids. Because of rapid symptom progression, he underwent resection of the left frontal lesion, which showed EBV-induced diffuse large B-cell lymphoma (DLBCL). Mycophenolate mofetil was discontinued, and within 24 h of one dose of intravenous 500 mg/m^2 rituximab, he had a dramatic improvement in left eye vision and memory loss. He experienced mixed responses to rituximab after 3 cycles. Following one dose of high-dose methotrexate, he developed subsequent chronic kidney disease and required dialysis. He received whole-brain radiation therapy with craniospinal radiation and is currently in complete remission. An EBV-induced DLBCL diagnosis should be highly considered for patients with periventricular lesions and EBV-positive cerebrospinal fluid. Misdiagnosis or delay in PCNSL diagnosis because of atypical features in disease presentation and radiographic findings could lead to PCNSL progression and worsening neurological deficits.

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare extranodal subtype of non-Hodgkin lymphoma (NHL) that affects the brain, leptomeninges, eyes, cranial nerves, and spinal cord; it accounts for ~3% of all primary brain tumors (1). Commonly, the underlying pathology of PCNSL is diffuse large B-cell lymphoma (2). PCNSL is most often seen as isointense or hypointense lesions on both T1- and T2-weighted MRI scans, and the lesions tend to moderately enhance with intravenous contrast administration (3).

Approximately 65% of PCNSLs present as a single brain lesion (4). Lesions most commonly affect the supratentorial brain and are typically found in contact with the ventricular and meningeal surfaces, but they can also lay deeper within the brain parenchyma (5). The disease typically does not involve the brainstem, cerebellum, or spinal cord. PCNSL incidence increases with age, with a peak incidence among those between 50 and 70 years old (6). Incidence also increases with congenital and acquired immunosuppression, and PCNSL is commonly mediated by Epstein-Barr virus (EBV), especially in the context of inadequate cytotoxic T-cell activity (7).

EBV-associated PCNSL is a known complication of iatrogenic immunosuppression in the posttransplant population and

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Abbreviations: CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; PCNSL, primary central nervous system lymphoma; PCR, polymerase chain reaction; VZV, varicella-zoster virus; WBRT, whole brain radiation therapy

Key words: primary CNS lymphoma, Epstein-Barr virus, immunosuppression, Myasthenia Gravis, whole brain radiation therapy, autoimmune disorder

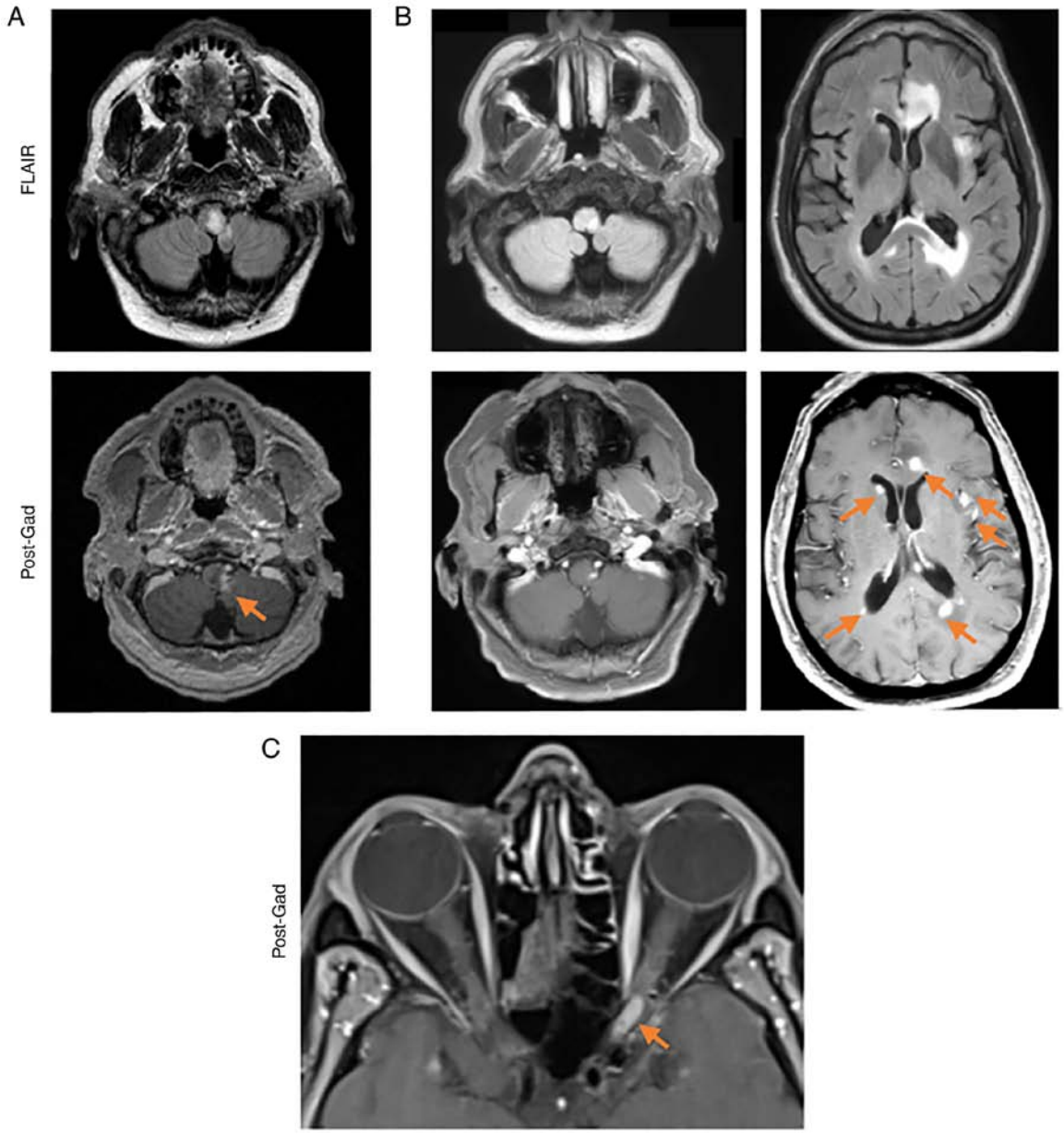

Figure 1. MRI findings. (A) Brain MRI at first presentation shows a solitary enhancing left medullary lesion. (B) Brain MRI with and without gadolinium 3 months after original presentation shows multifocal T2 FLAIR changes with associated nodular enhancement in bilateral periventricular white matter with involvement of corpus callosum, cerebellum, cerebrum and brainstem. (C) Orbital MRI with and without gadolinium 3 months after original presentation shows the enhancing left optic nerve lesion. FLAIR, fluid-attenuated inversion recovery; Post-Gad, post-gadolinium.

among those receiving immunosuppressive therapy for immune-mediated conditions (8). Withdrawal of the immunosuppressive agent can even lead to PCNSL regression for some patients (9). Though vitrectomy or cerebrospinal fluid (CSF) cytology may be sufficient to diagnose PCNSL if the eye is involved, the gold standard for diagnosis is a stereotactic biopsy (4).

In this article, we report a case of EBV-induced PCNSL caused by prolonged immunosuppression with mycophenolate mofetil in which the patient experienced delays in diagnosis because of atypical features in the disease presentation and radiographic findings. This case report highlights how misdiagnosis or delayed PCNSL diagnosis and delayed withdrawal of immunosuppressive agents can lead to PCNSL progression. This patient experienced remission after whole-brain radiation therapy (WBRT).

Case report

The patient was a 68-year-old right-handed man with stage 3 chronic kidney disease and acetylcholine receptor antibody-positive myasthenia gravis (MG) who received mycophenolate mofetil 500 mg twice daily for 22 years. He presented with a few months of worsening left eye vision and left facial paresthesia and several weeks of imbalance, vertigo, sensory changes, and vocal changes. He initially attributed his declining left eye vision to a herpes zoster ophthalmicus infection that occurred 3 months prior to presentation, in the left V1 distribution. For this infection, he was previously prescribed antiviral medication.

On examination, he was found to have mild left eye ptosis; his eyelid was above the level of his pupil, which was not

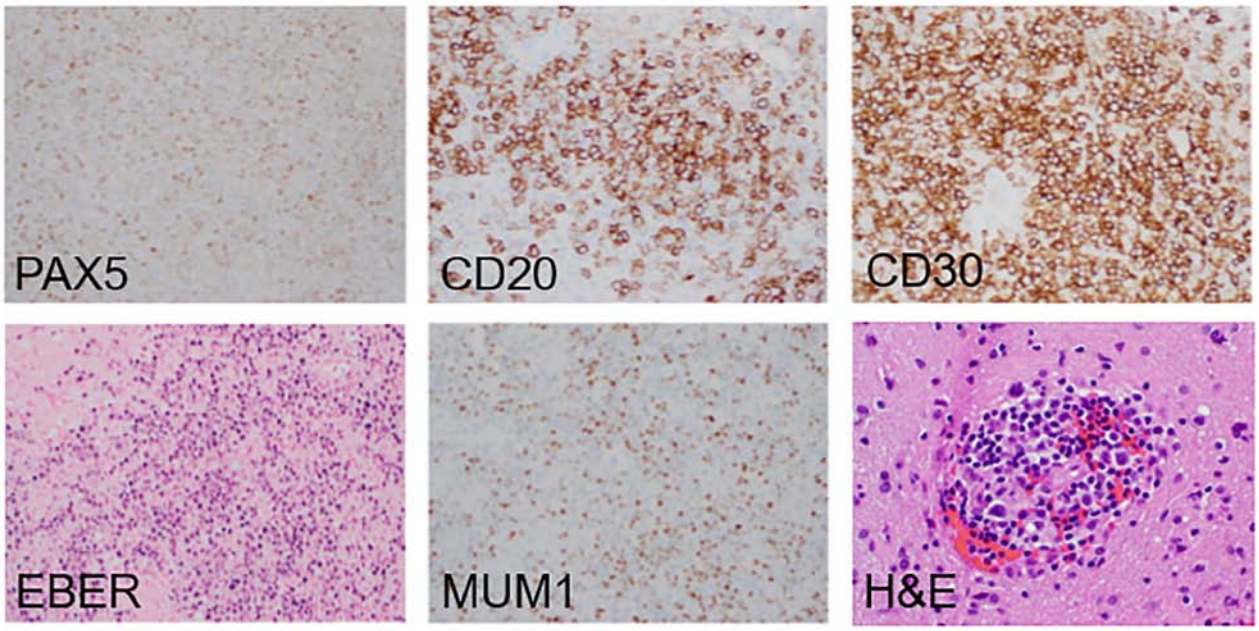

Figure 2. Pathology findings. The left frontal lesion showed gliosis, microglial activation, mild chronic inflammation and only scattered atypical cells. H&E staining showed that neoplastic cells possessed small volumes of eosinophilic cytoplasm, with indiscernible cell borders and oval pleomorphic nuclei with occasional nucleoli. Immunostaining showed positive expression of PAX5, CD20, MUM1 and CD30. *In situ* hybridization was positive for EBER. PAX5, paired box 5; MUM1, interferon regulatory factor 4; EBER, Epstein-Barr virus-encoded small RNAs; H&E, Hematoxylin and eosin.

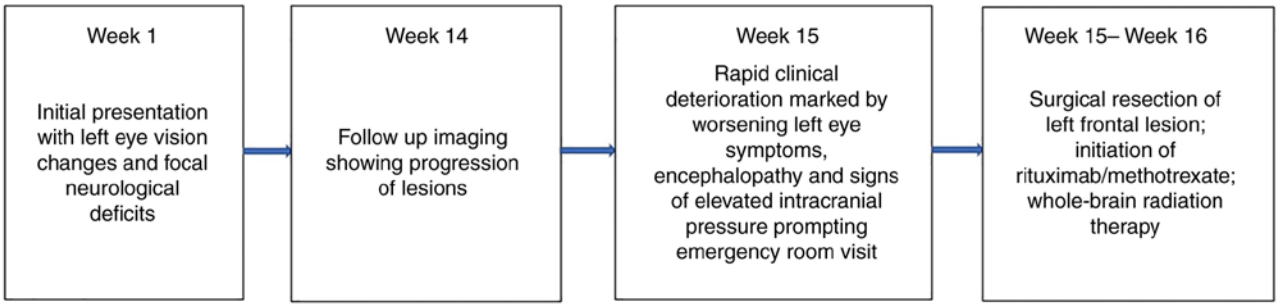

Figure 3. Summary of timeline of clinical symptoms, findings and treatment.

fatigable. He had decreased left eye vision (20/40); restriction of left eye abduction and adduction; restriction of right abduction without subjective diplopia; vertical and horizontal nystagmus; slight asymmetry of the left lower face; decreased sensation of soft touch in the left lower extremity; truncal and left-sided ataxia; and left-sided hyperreflexia. On ophthalmological examination, he was found to have punctate keratitis with potential endotheliitis.

A brain MRI showed a suspicious enhancing lesion within the left medulla that extended to the cerebellum, with no evidence of hydrocephalus (Fig. 1A). An MRI of the spine and CT scan of the chest, abdomen, and pelvis did not show any malignancy. A lumbar puncture (LP) showed an opening pressure of 27 cm H_2O, a nucleated cell level of 238 cell/mm^3 with 98% lymphocytes, and a total protein level of >200 mg/dl. CSF was negative for any bacterial or fungal cultures. CSF analysis was negative for carcinoma, meningitis, herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Toxoplasma gondii parasite, Lyme disease, or venereal disease research laboratory (VDRL) tests; however, the patient was positive for EBV by qualitative polymerase chain reaction (PCR). At this point, the working diagnosis for this patient was VZV vasculopathy. He received 750 mg of intravenous acyclovir twice daily for 14 days and 60 mg of oral prednisone for 5 days.

Three months after his discharge, a repeat brain MRI showed multiple new enhancing lesions that developed bilaterally along the periventricular white matter with involvement of the corpus callosum. There were several lesions in peripheral locations of the cerebrum, cerebellum, and brainstem with little mass effect (Fig. 1B) and no evidence of restricted diffusion. He was referred to the Neuro-Oncology clinic at Moffitt Cancer Center (MCC). During his first visit at MCC, he complained of blurry vision and pain in the left eye, paresthesia involving the left side of his face, poor balance, and left-sided ataxia. An orbital MRI showed an enhancing lesion on the left optic nerve and full-thickness enhancement (Fig. 1C). At this time, his wife also reported that he exhibited intermittent confusion, memory decline, and word-finding difficulty that had been more noticeable in the last few months.

Table I. Clinical summary.

Characteristics	Case
Clinical features at initial presentation	Left eye vision changes and left facial paresthesias for a few months with several weeks of imbalance, vertigo, sensory changes and vocal changes
MRI results at initial presentation	Abnormal T2-weighted-FLAIR contrast-enhancing lesion within the left medulla extending to the cerebellum without evidence of hydrocephalus
CSF results at initial presentation	Nucleated cells: 238 cell/mm^3 with 98% lymphocytes; total protein level: >200 mg/dl; negative bacterial and fungal cultures, herpes simplex virus DNA by qualitative PCR, varicella-zoster virus DNA by PCR, cytomegalovirus DNA by PCR, Toxoplasma gondii DNA by PCR, Lyme disease DNA by PCR, venereal disease research laboratory test; cytology was negative for the presence of atypical/malignant cells; positive for EBV by PCR
Therapies given at initial presentation	750 mg of intravenous acyclovir twice daily for 14 days and oral prednisone 60 mg daily for 5 days
MRI results at 3-months follow up	Multiple new T2-weighted FLAIR contrast-enhancing lesions bilaterally along the periventricular white matter with involvement of the corpus callosum and several lesions in peripheral locations of the cerebrum, cerebellum and brainstem with little mass effect
Clinical features at second presentation	Encephalopathy with acutely worsening blurry vision and pain in the left eye, nausea, and vomiting
CSF results at second presentation	Nucleated cells: 54 cells/mm^3 with 88% lymphocytes; total protein level: 153 mg/dl. Negative bacterial and fungal cultures; cytology was negative for the presence of atypical/malignant cells; positive for EBV by PCR with qualitative analysis revealing 201,000 virus copy/cc
Interventions and therapies given at second presentation	Gross total resection of the left frontal lesion which showed EBV-induced diffuse large B-cell lymphoma (see Fig. 2); intravenous dexamethasone; 3 cycles of 500 mg/m^2 intravenous rituximab; whole-brain radiation therapy with craniospinal radiation
Response to treatment	Dramatic improvement in his left eye vision and cognitive deficits followed by complete remission

FLAIR, fluid-attenuated inversion recovery.

Two days after his visit, he presented to his local emergency room with acutely worsening blurry vision and pain in the left eye, nausea, and vomiting. He was prescribed 4 mg of dexamethasone every 6 h and received 3 doses before he was transferred to Moffitt Cancer Center. A repeat brain MRI showed interval increases in the size of his preexisting lesions, with interval development of central necrosis, restricted diffusion, more indistinct margins of the enhancement, and areas of petechial hemorrhage. A repeat LP showed an opening pressure of 15 cm H_2O, a nucleated cell level of 54 cells/mm^3 with 88% lymphocytes, and a total protein level of 153 mg/dl. CSF analysis was again negative for carcinoma and positive for EBV via quantitative PCR, with 201,000 virus copies/cc. A repeat CT scan of the chest, abdomen, and pelvis was negative for malignancy.

A week later, a neurosurgeon resected the left frontal lesion, which was identified as EBV-induced diffuse large B-cell lymphoma. The lesion showed positive expression of PAX5, CD20, MUM1, CD30, and *EBER* (Fig. 2). Peripheral blood mononuclear cells (PBMC) were prepared by Ficoll-Hypaque density gradient separation. Automated counts (Sysmex KX-21N; Sysmex, Lincolnshire, IL) were performed to quantify recovered cells. DNA was isolated from PBMC ($2x10^6$ cells) and plasma (200 μl) using QIAmp DNA blood mini reagents (Qiagen, Gaithersburg, MD). EBV qPCR was performed on DNA using Qiagen/Artus EBV analyte specific reagents (Qiagen) and 7500 Real-Time PCR System (Applied Biosystems/Thermo Fisher Scientific, Foster City, CA), with a 97 base pair region of EBV nuclear antigen-1 (EBNA-1) as the target. The primer used for EBV PCR testing in CSF was *EBNA-1 reaction*: 5'CCGCTCCTACCTGCAATATCA 3' (forward primer) and 5'GGAAACCAGGGAGGCAAATC 3' (reverse primer); 5'VIC-TGCAGCTTTGACGATGG-MGB 3' (probe). The limit of detection by qPCR was 50 copies per ml plasma or per 100,000 PBMCs. Mycophenolate mofetil was discontinued. The patient received one 500 mg/m^2 dose of intravenous rituximab and experienced a dramatic improvement in his left eye vision and cognitive deficits within 24 h. He also received one dose of high-dose methotrexate (3.5 gm/kg) the next day but unfortunately developed worsening kidney

failure, which required hemodialysis. He received 5 more doses of rituximab on a 2-week schedule (ie, treated for a total of 12 weeks), and a repeat brain MRI after the sixth dose showed improvement in some lesions and progression in others. He received WBRT (23.4 Gy) with craniospinal radiation, which resulted in complete remission. Please refer to Fig. 3 for a gross visual summary of the timeline of events in the present case and to Table I for a more in-depth summary of the timeline of clinical symptoms, findings and treatments.

Discussion

This case report highlights how delays in PCNSL diagnosis due to atypical imaging features can lead to PCNSL progression. It also emphasizes the importance of recognizing diagnostic clues, such as positive EBV PCR, the patient's immunosuppression, presentation with progressive neurological symptoms, and presence of a new brain lesion. Early diagnosis and treatment of PCNSL among patients receiving immunosuppressive therapy is crucial; in this case, a heightened index of suspicion for PCNSL based on these factors would have warranted a brain biopsy during the patient's first hospitalization. However, the patient had only a medullary lesion, which was thought to be due to VZV vasculitis at presentation, so a brain biopsy was not performed. An earlier diagnosis would have resulted in immediate treatment of the isolated brain lesion and, subsequently, reduced neurological burden.

This case had limitations that complicated the diagnostic process. Firstly, a notable limitation of this patient's diagnosis was his recent co-occurring zoster infection at the time of his initial presentation. It was thought that his brain lesion in the medulla was due to VZV vasculopathy. Secondly, PCNSL is known to be notably responsive to steroids, so the patient's dexamethasone treatment may have masked some of his neurological symptoms and normalized the cellular examination of the CSF. Regarding the CSF studies, it is also notable that we did not check CSF interleukin-10 for this patient which is a known helpful tool to distinguish between an infection and B cell lymphoma. Thirdly, there is a lack of standardized association between EBV-induced PCNSL and neurological decline among immunosuppressed patients. However, there are extensive case reports and series that highlight presence of EBV-induced PCNSL in patients with immune disorders who are receiving immunosuppressive agents (8-15). Medications such as mycophenolate mofetil causes further immunosuppression, which can increase the risk of viral infections, including EBV, which then increases the risk of developing PCNSL. Still, the causal relationship between prolonged exposure to mycophenolate mofetil and PCNSL is not well established. Our patient had multiple risk factors for EBV-induced PCNSL, including advanced age, immunosuppressive medications, and presence of MG.

Future research studies could be conducted in with patients with EBV-induced PCNSL to systematically correlate the presence and type of immunosuppressive therapy these patients have received. This will help prevent delays in the identification of EBV-induced PCNSL in both primary and acute care services. It will also reinforce the need to expeditiously escalate diagnostic workup to a stereotactic brain biopsy if clinical suspicion for PCNSL is high. Instituting early treatment with rituximab and methotrexate with or without WBRT may improve patients' survival, morbidity, and quality of life.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

OB, SSK and SM designed and conceptualized the study, acquired and analyzed the data, and drafted the manuscript for intellectual content. GW, DI, TR, AE and YP interpreted the data and revised the manuscript for intellectual content. RM reviewed the brain biopsy pathology and provided the pathology slides. All authors read and approved the final manuscript. SM and OB confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This publication is considered exempt from Institutional Review Board review at Moffitt Cancer Center, as the retrospective review of records for publication of a single-patient case report is not considered to be research involving human subjects.

Patient consent for publication

Written informed consent was obtained from the patient/patient representative for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal. Institutional approval was not required to publish the case details.

Competing interests

The authors declare that they have no competing interests.

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