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COMPLEMENT-ASSOCIATED NEURONAL LOSS IN A PATIENT WITH CASPR2 ANTIBODY-ASSOCIATED ENCEPHALITIS

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Recently, CASPR2 antibody–associated encephalitis was presented as a subentity of encephalitis with antibodies against the voltage-gated potassium channel (VGKC) complex. Besides limbic encephalitis, CASPR2 antibodies are also found in Isaac syndrome and Morvan syndrome.[1,2] The specific underlying pathogenic mechanisms in limbic encephalitis are unknown. Our data suggest an antibody- and complement-mediated pathology in CASPR2 antibody–associated encephalitis.

Since 2010, our 62-year-old male patient has had unrecognized dyscognitive seizures and acoustic hallucinations. In 2012, a loss of short-term memory was also noticed. EEG and MRI conducted in August 2012 were normal (figure, A). In January 2013, his symptoms worsened and he was admitted to a hospital. At this time, MRI examination showed volume and T2/fluid-attenuated inversion recovery (FLAIR) signal increase in the right hippocampus and amygdala (figure, A).

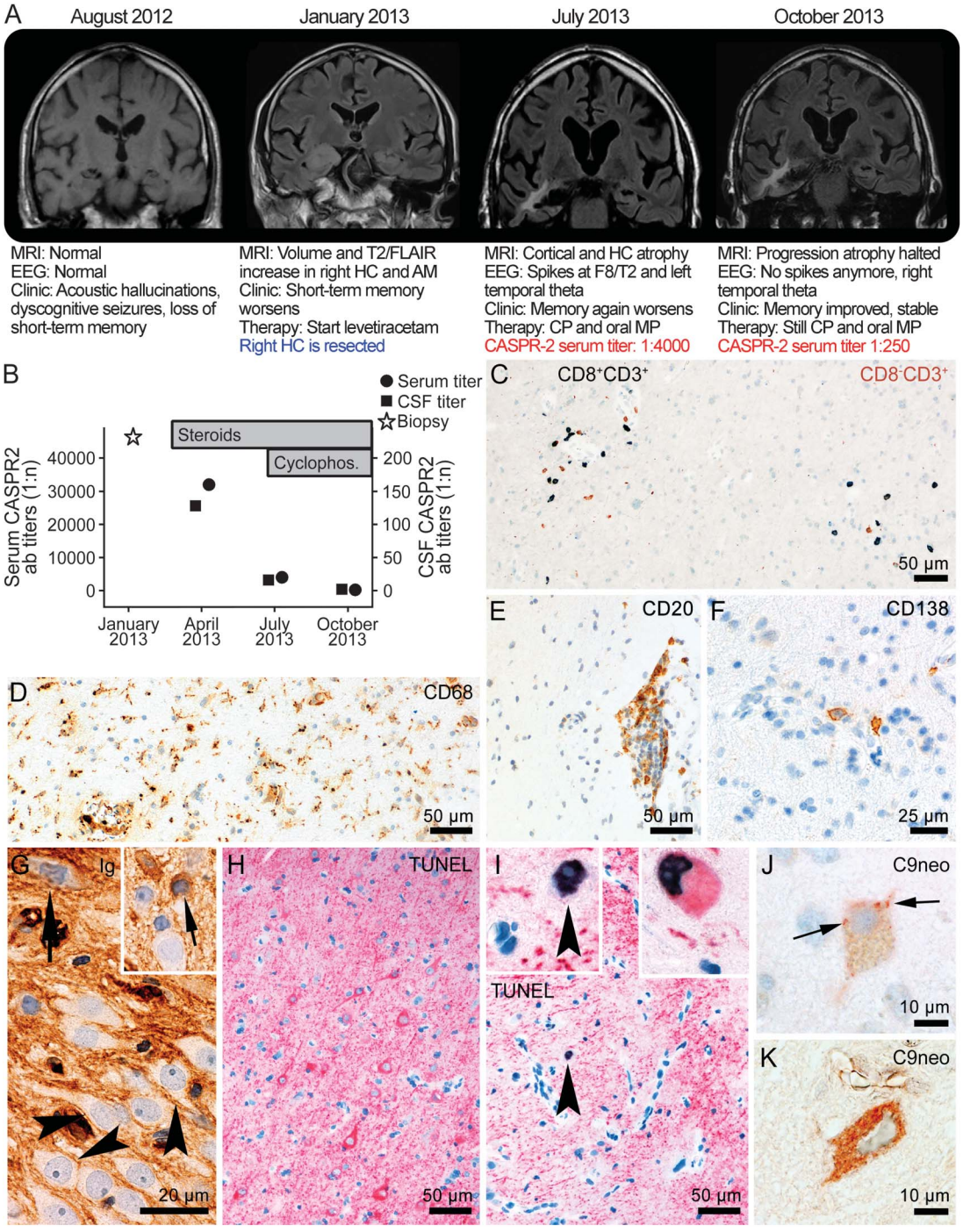
Due to suspicion of a malignant process, the right hippocampus and parts of the temporal lobe, including the amygdala, were resected. Neuropathologic investigations ruled out tumor formation. Detailed evaluation, however, showed moderate parenchymal presence of $CD3^+$ and $CD8^+$ T lymphocytes (figure, C). The lack of these cells in apposition to neurons suggests the absence of T cell–mediated neuronal destruction. CD68 staining showed mild activation of microglial cells (figure, D). Furthermore, CD20 and CD138 immunostaining showed small numbers of B cells (figure, E) and plasma cells (figure, F), predominantly in the perivascular space of blood vessels. Immunoglobulin (Ig) staining showed a strong leakage of Ig through the blood-brain barrier. In areas with less blood-brain barrier leakage, however, some deposition of Ig on the membranes of neurons could be detected (figure, G). In these areas some neurons showed shrinkage and nuclear changes, suggesting degeneration (figure, G). Degenerating neurons were absent in the amygdala (figure, H), but the hippocampus revealed few degenerating neurons (figure, I). Complement deposition, like in hippocampi of other patients with VGKC-complex encephalitis,[3] was detected in a few neurons (figure, J and K). Such deposition was not found in the cortex or hippocampi of NMDAR−, Hu−, Ma2−, or GAD encephalitis patients, patients with mesial temporal lobe epilepsy with hippocampal sclerosis or Alzheimer disease, or normal controls.[3]

In March 2013, VGKC-complex antibody–associated encephalitis was diagnosed and methylprednisolone (MP) treatment was started (IV followed by oral administration). This resulted in a remarkable improvement of short-term memory. To detect a potential underlying malignancy, we then performed a whole-body fluorodeoxyglucose PET, which was negative. In July 2013, 2 months after the last MP IV treatment, the patient's short-term memory problems again worsened, while attentional deficits fluctuated. MRI scans now showed a progressive cortical and hippocampal atrophy (figure, A). At this time, serum and CSF were analyzed again and were found to be positive for CASPR2 antibodies (serum and CSF obtained in April, titers 1:32,000 and 1:128, respectively, see panel B of the figure). There were no antibodies to LGI1, NMDAR, GAD65, $GABA_B$ receptors, AMPA receptors type 1 and type 2, or glycine receptors.

The antibody index (AI, i.e., the ratio between the CSF/serum quotients of CASPR2 antibody titers and total IgG concentrations) determines whether antibodies are formed intrathecally. We considered an AI >4 as an indication of intrathecal production of the specific antibody. This conservative cutoff was used because any lower cutoff may give false positive results when titers are used.[3] The values were as follows: April 2013: AI = 1.1; July: AI = 1.0; and October: AI = 2.5. Therefore, CASPR2 antibodies were produced mainly peripherally.

In July 2013, in addition to oral prednisolone, monthly cyclophosphamide infusions (15 mg/kg body weight) were started (figure, A). Thereafter, CASPR2 antibodies decreased (figure, B), neuropsychological deficits vanished, and the patient's condition stabilized. Further CSF analysis showed no pleocytosis or intrathecal IgG synthesis. MRI showed a discrete FLAIR intense signal in the left

Figure **Disease course and neuropathology of CASPR2 encephalitis**



(A) Summary of the most important changes during the disease course. Shown are consecutive cerebral fluid-attenuated inversion recovery (FLAIR) MRI scans in relation to therapeutic treatment, clinical symptoms, and CASPR2 titer. The swollen hippocampus (HC) and amygdala (AM) were resected in January 2013. Note the progressive global atrophy of the brain (from August 2012 to July 2013), which largely stopped in October 2013. The left HC, however, remained FLAIR intense in October 2013. (B) Changes in CASPR2 antibody (ab) titers in serum (dots) and CSF (squares) during the disease course. The graph shows a decline of the titers in serum and CSF after prolonged administration of cyclophosphamide and oral prednisolone (indicated by the gray bars on top). The star indicates the time point of the biopsy. (C–K) Neuropathology of the right hippocampus. (C) Double staining for CD3 and CD8 shows moderate numbers of T cells ($CD8^+$ T cells are blue, $CD3^+CD8^-$ [$CD4^+$] T cells are brown). (D) CD68 staining shows moderately activated microglial cells. (E) Staining for CD20 shows the presence of B cells in a perivascular cuff. (F) Staining for CD138 shows the presence of some plasma cells. (G) Staining for immunoglobulins (Ig) reveals strong leakage into the parenchyma. Deposition of immunoglobulin on neuronal membranes is indicated by the arrowheads. The arrow in the upper corner points at a degenerating neuron with nuclear changes. The inset shows an additional degenerating neuron (arrow) with a condensed nucleus. (H) Staining for TUNEL (black) and MAP2 shows the absence of degenerating cells in the amygdala of this patient. (I) In the hippocampus, a single TUNEL-positive neuron is indicated by the arrowhead. The insets show an enlargement of this neuron (left side) and a second MAP2+ TUNEL+ neuron. (J, K) Staining for complement C9neo (end complex) reveals a neuron with minor deposition, depicted by arrows (J), and a neuron with major deposition (K). CP = cyclophosphamide; MP = methylprednisolone.

hippocampus but no further progression of global atrophy (figure, A). We had no indications for neuromyotonia or myasthenia gravis as CASPR2-associated symptoms at any time.

The case study presented here can refine our knowledge of CASPR2 antibody–associated encephalitis. We found signs of an antibody- and complement-mediated inflammation, which is consistent with our earlier observation in VGKC-complex encephalitis.[4] These findings are in contrast to NMDAR encephalitis, in which no evidence of complement deposition was found.[5,6] This is the first description of neuronal complement activation in a patient with CASPR2 encephalitis. The features correlate well with the MRI-documented hippocampal and cerebral atrophy. In our case, cyclophosphamide combined with oral prednisolone induced a partial remission. This partial remission was, as described earlier,[7] correlated with a parallel decline of serum and CSF antibodies. Therefore, clinical deficits in this condition seem to be related to 2 encephalitis-related processes: (1) a destructive, complement-mediated process leading to irreversible brain tissue loss, and (2) a direct, antibody-mediated functional process that can be stopped by removal of antibodies from CSF and serum and that results in an almost immediate clinical improvement.

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