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Hit by a Smooth CD8: T-Cell Attack on Hippocampal Neurons Triggers Limbic Encephalitis and Epilepsy

CD8 + T-Lymphocyte-Driven Limbic Encephalitis Results in Temporal Lobe Epilepsy

Pitsch J, Loo KMJ, Gallus M, et al. Ann Neurol. 2021;89(4):666-685.

Objective: Limbic encephalitis (LE) comprises a spectrum of inflammatory changes in affected brain structures including the presence of autoantibodies and lymphoid cells. However, the potential of distinct lymphocyte subsets alone to elicit key clinicopathological sequelae of LE potentially inducing temporal lobe epilepsy (TLE) with chronic spontaneous seizures and hippocampal sclerosis (HS) is unresolved. Methods: Here, we scrutinized pathogenic consequences emerging from $CD8^+$ T cells targeting hippocampal neurons by recombinant adeno-associated virus-mediated expression of the model-autoantigen ovalbumin (OVA) in CA1 neurons of OT-I/RAG1−/− mice (termed "OVA-$CD8^+$ LE model"). Results: Viral-mediated antigen transfer caused dense $CD8^+$ T cell infiltrates confined to the hippocampal formation starting on day 5 after virus transduction. Flow cytometry indicated priming of $CD8^+$ T cells in brain-draining lymph nodes preceding hippocampal invasion. At the acute model stage, the inflammatory process was accompanied by frequent seizure activity and impairment of hippocampal memory skills. Magnetic resonance imaging scans at day 7 of the OVA-$CD8^+$ LE model revealed hippocampal edema and blood-brain barrier disruption that converted into atrophy until day 40. $CD8^+$ T cells specifically targeted OVA-expressing, SIINFEKL-H-2Kb -positive CA1 neurons and caused segmental apoptotic neurodegeneration, astrogliosis, and microglial activation. At the chronic model stage, mice exhibited spontaneous recurrent seizures and persisting memory deficits, and the sclerotic hippocampus was populated with $CD8^+$ T cells escorted by NK cells. Interpretation: These data indicate that a $CD8^+$ T-cell-initiated attack of distinct hippocampal neurons is sufficient to induce LE converting into TLE-HS. Intriguingly, the role of $CD8^+$ T cells exceeds neurotoxic effects and points to their major pathogenic role in TLE following LE.

Commentary

Temporal lobe epilepsy (TLE) is a common type of epilepsy characterized by focal seizures that can be drug-resistant.[1] In TLE, the hippocampal and amygdala brain regions are typically damaged and, as a result, cognitive deficits often develop along with recurrent unprovoked seizures.[1,2] Severe neuronal loss, gliosis, and inflammation in the hippocampus are some of the neuropathological hallmarks of TLE. These can result as a consequence of brain injuries from events such as trauma, stroke, and status epilepticus (SE), as well as from limbic encephalitis (LE).[3] LE results from the inflammation of the medial temporal lobe and limbic areas of the brain provoked by the production of autoantibodies that target one's own cells or tissues.[3,4] Autoimmune LE with spontaneous seizures is associated with antibodies against glutamic acid decarboxylase 65 (GAD65), the gamma-amino-butyric B receptor (GABABR), and the N-methyl-D-aspartate receptor (NMDAR), among others.[3-5] Interestingly, LE is associated with new onset TLE in adults[6]; though how exactly LE leads to the development of TLE is not definitively known. While the presence of autoantibodies is well known to be a primary trigger for inflammatory responses responsible for LE, less is known on the role that the accompanying T-cell infiltrates have on the neuropathology and pathophysiology of LE and TLE.

In the study by Pitsch et al[6] recently published in Annals of Neurology, a mouse model of LE (OVA-$CD8^+$ LE) was developed to determine if a $CD8^+$ T-lymphocyte driven attack that specifically targets excitatory hippocampal neurons, rather than an autoantibody response, is sufficient to cause unprovoked seizures and cognitive decline along with the neuropathology typically seen in TLE. $CD8^+$ T lymphocytes are part of the adaptive immune system that becomes activated via major histocompatibility complex class I (MHCI) peptides on the surface of infected cells. Most $CD8^+$ T-cells are cytotoxic in the effector mode and kill targeted cells. Thus, to achieve this effect on hippocampal neurons, the authors used T-cell receptor (TCR) transgenic (OT-1) mice deficient in the recombination activating gene 1 (RAG1) (denoted as OT-1 mice). All peripheral T-cells in these mice specifically recognize the restricted ovalbumin (OVA) for chicken peptide SIINFEKL (OVA 257-264), when presented by MCHI. To incite a MCHI-dependent $CD8^+$ T-cell driven attack on hippocampal neurons, recombinant adeno-associated virus (rAAV) encoding for synapsin-driven

OVA-SIINKFEL (rAAV-OVA) or control (GFP) proteins was injected bilaterally into the CA1 hippocampal area of adult mice. Therefore, only the cells that received the specific instructions to transcribe the SIINFEKL peptide were targeted by the $CD8^+$ T-cells. These were compared to C57Bl6/N mice with similar rAAV injections as the OT-1 mice.

This is an interesting model that recapitulates the pathophysiology seen in models of acquired epilepsy generated by electrically or chemically-induced SE.[7] The rAAV injections encoding the SIINFEKL peptide in hippocampi of OT-1 mice provoked the development of interictal activity and spontaneous seizures that were first evident at 4 days, and were more frequent at 5 days after rAAV-OVA injection. Seizures were frequent in the first week (~13.8 seizures/week) but declined substantially (~.5 seizures/week) by weeks 7 and 8. Despite the drastic decline, these animals developed long-lasting cognitive deficits that were present at both 1 and 7 weeks after rAAV-OVA injections. The initial seizure spike suggests the possibility that these seizures themselves may further disrupt the neuronal circuitries to potentiate epileptogenic processes, similar to SE-induced TLE models.[7] It is intriguing that the seizure frequency decreased over time in this model because it contrasts with the increase in seizure severity typically found in chemoconvulsant models of SE and acquired TLE.[7] This could be due to differences in the extent of injury in this LE model vs the kainate or pilocarpine models, which may produce variable latent periods for the generation of epileptic circuitries. Because the study was limited to 8 weeks after the rAAV-OVA delivery, the full impact that the $CD8^+$ T-cell "attack" on CA1 hippocampal neurons has on chronic epilepsy is yet to be determined.

For a successful $CD8^+$ T-cell driven attack on OVA-SIINKFEL positive hippocampal neurons, these immune cells must migrate to the hippocampus. The study shows that the infiltration of $CD8^+$ T-cells into the brain occurred alongside progressive increases in other peripheral immune cells such as natural killer cells, neutrophils, and monocytes. However, it is not clear what directly triggered the blood brain barrier (BBB) disruption that would allow the extravasation of these cells into the brain. The evidence presented confirms BBB leakage through the presence of albumin in the hippocampus at 5 days following the rAAV-OVA injection, when the seizure frequency was highest. This suggests that the seizures per se may be causal to BBB disruption and the accompanying migration of peripheral cells and increases in pro-inflammatory cytokines, which can further aggravate the neurodegeneration in the hippocampus. Nevertheless, selective expression of SIINFEKL by CA1 neurons, and their association with the $CD8^+$ T-cells, indicates they were preferentially targeted as early as 2 days after the rAAV-OVA injection. This evidence suggests that the $CD8^+$ T-cells may have triggered the initial neurodegeneration that led to the subsequent development of spontaneous recurrent seizures and the TLE pathology.

The neuropathology of the OVA-$CD8^+$ LE mice closely resembled that of the kainate or pilocarpine-induced animal models of TLE. This suggests that common primary mechanisms that trigger seizures in these experimental models—chemoconvulsants or directed $CD8^+$ T-cell attack—may underlie secondary pathogenesis including gliosis, BBB disruption, peripheral immune cell infiltration, inflammatory cytokine release, and synaptic network remodeling. In addition to the infiltration of peripheral immune cells, the loss of hippocampal CA1 neurons correlated with microgliosis and astrogliosis between 3 and 28 days after rAAV-OVA injection. The spatiotemporal profile of these cellular changes is similar to those found following SE events, which also parallel seizure generation and cognitive decline.[7-9] These findings support that immune and inflammatory events are comparable across preclinical models of TLE. However, a limitation is that these pathological parallels make it difficult to interpret which processes may play a major mechanistic role in the epileptogenic hippocampal remodeling. To narrow down the main contributors to the epilepsy pathology, it would be valuable to interrogate in detail the temporal evolution of the cellular and molecular changes that occur from viral delivery to the initiation of seizures in the OVA-$CD8^+$ LE mice. In addition, quantitative analyses of the neuropathological alterations throughout this study could have provided some objective correlational data useful to determine the extent to which the severity of seizures at 1 week after rAAV-OVA injection is associated with the neuropathology in each animal.

In summary, Pitsch et al developed a novel mouse model of OVA-$CD8^+$ LE that reproduced the peripheral immune cell infiltration, gliosis, and neuroinflammation in the hippocampus that is representative of human LE. The authors demonstrated that a $CD8^+$ T-cell-mediated loss of CA1 hippocampal neurons is sufficient to produce an epileptic brain with characteristics of TLE. An advantage of novel mouse models of epilepsy such as the OVA-$CD8^+$ LE mice is that it provides us with new tools to further investigate how the development and progression of LE results in chronic epilepsy. In addition, this model can be useful to interrogate how severe immune responses and inflammatory conditions modify specific brain areas, such as the hippocampus, to promote hyperexcitable circuits that result in epilepsy and memory deficits. While animal models do not fully reproduce the neuropathology or pathophysiology of human epilepsy, they are excellent tools to investigate the mechanisms underlying disease development and progression. This, in turn, is critical for the identification and development of successful therapeutic treatments for epilepsy.

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