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Exploring CITEs of Inflammation in the Human Epilepsy Brain

Single-Cell Transcriptomics and Surface Epitope Detection in Human Brain Epileptic Lesions Identifies Pro-Inflammatory Signaling

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Epileptogenic triggers are multifactorial and not well understood. Here we aimed to address the hypothesis that inappropriate pro-inflammatory mechanisms contribute to the pathogenesis of refractory epilepsy (non-responsiveness to antiepileptic drugs) in human patients. We used single-cell cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) to reveal the immunotranscriptome of surgically resected epileptic lesion tissues. Our approach uncovered a pro-inflammatory microenvironment, including extensive activation of microglia and infiltration of other pro-inflammatory immune cells. These findings were supported by ligand-receptor (LR) interactome analysis, which demonstrated potential mechanisms of infiltration and evidence of direct physical interactions between microglia and T cells. Together, these data provide insight into the immune microenvironment in epileptic tissue, which may aid the development of new therapeutics.

Commentary

Tissue inflammation is a key component of many diseases, including epilepsy. A striking example is Rasmussen's encephalitis—a rare neurological disorder characterized by unilateral inflammation of the cerebral cortex and drug-resistant epilepsy.[1] The inflammation is thought to be driven by microglia and T-cells with possible contribution of autoantibodies against a variety of neural tissue-specific antigens, such as GluR3, alpha-7 nicotine acetylcholine receptor, and Munc-18-1.[1] While Rasmussen's encephalitis is an unusual cause of epilepsy, it has been suggested that up to 35% of refractory epilepsies of unknown etiology involve immunological mechanisms.[2] Antibody-mediated limbic and extralimbic encephalitis, multisystem inflammatory disorders, West syndrome, and Landau-Kleffner syndrome are all examples of inflammatory conditions linked to recurrent seizures.[3] Many focal epilepsies, such as mesial temporal lobe epilepsy and cortical epilepsies associated with dysplastic lesions, also exhibit signs of neuroinflammation, as evidenced by proliferation of reactive astrocytes and microglia, increased expression of pro-inflammatory cytokines and chemokines, and blood–brain barrier abnormalities with infiltration of circulating immune cells.[4]

However, despite decades of research, there remains a remarkable lack of randomized, controlled trials of immune-modulating therapies for epilepsy. Most treatments are anecdotal and based on personal and institutional preferences and typically involve "shotgun" approaches, such as plasmapheresis and administration of corticosteroids and intravenous immunoglobulins.[2] Important barriers to progress include differences in immune mechanisms between rodent models and humans, the idea that the brain immune system is separated and different from the systemic immune system, and the complexity and heterogeneity of the brain versus more commonly studied immune-organs, such as blood, lymph nodes, and spleen.

The present work by Kumar et al[5] overcomes many of these barriers by investigating brain and systemic immune perturbations in pediatric patients with drug-resistant focal epilepsy and concurrent brain lesions, using state-of-the-art single cell transcriptomic and proteomic technologies combined with a systems biology analytical approach. One of the approaches they used is CITE-seq (**C**ellular **I**ndexing of **T**ranscriptomes and **E**pitopes by **seq**uencing), which involves RNA sequencing and surface antigen mapping at single-cell resolution. When combined with network-biology based approaches, the CITE-seq data can be used to reveal complex, ligand-receptor relationships between cells. The authors included surgically resected brain tissue (total of 11 samples) from 6 pediatric patients with drug-resistant epilepsy and postmortem brain tissue samples from people without neurological disorders.

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The authors first used CITE-seq to define resident and infiltrating immune cells in the epilepsy brain. They isolated over 85 000 cells and 22 000 genes and clustered the cell types based on gene expression levels and surface epitope content. Among the immune cells, which were identified through the expression of CD45 on the cell surface, 13 clusters were identified as microglia and 6 clusters were identified as infiltrating immune cells. A variety of nonimmune (CD45-negative) cells were also present and identified as oligodendrocytes and cells specific to the neurovascular unit. Notably, infiltrating immune cells were observed in all 11 tissue samples from the olfactory, frontal, or temporal lobes.

The microglia exhibited a pro-inflammatory phenotype in the epilepsy brain as evidenced by increased expression of genes for cytokines and chemokines, such as IL1b, IL1A, TNF, CLL2, and CCL4. In contrast, microglia from non-neurological control brains and from individuals with autism spectrum disorders did not overexpress these genes. Further, when comparing epilepsy brain tissue from the lesion versus the surrounding tissue, the lesion tissue had relatively higher levels of pro-inflammatory cytokines and chemokines. The widespread expression of pro-inflammatory genes had unexpected similarities with microglial expression patterns found in brain lesions in patients with multiple sclerosis such as increased expression of HLA-DRA and HLA-DPB1 and decreased expression of CX3CR1 and P2RY12. These findings raise the possibility that immune modulating drugs with effect on multiple sclerosis may also be effective treating certain epilepsies.

Using multispectral Opal dye 7 color immunohistochemistry imaging analysis, the IL1b expressing cells in the epilepsy brain were identified as allograft inflammatory factor 1 positive microglia and glial fibrillary acidic protein-positive astrocytes. Moreover, the IL1b-producing microglia showed reduced expression of the purinergic receptor P2RY12, which responds to neuronal activation by sensing ATP released by activated neurons and astrocytes. There is increasing evidence from other studies that adenosine and the purinergic system play important roles in epileptogenesis, and that pharmacological manipulation of the purinergic pathway may be used to treat epilepsy.[6] The data from the present study suggest that immune-mediated epilepsies may also involve potentially treatable purinergic mechanisms.

Further, the authors identified a potential mechanism of lymphocyte infiltration in the brain and found physically interacting T cells with microglia. Using a novel method—sequencing of physically interacting cells—the authors showed enhanced pro-inflammatory function in both the physically interacting microglia and T cells, compared to cells not in direct interaction. Additionally, flow cytometry analysis of peripheral blood from patients with epilepsy revealed an imbalance toward pro-inflammatory T cell subsets and a marked IL-17 signature, as previously reported by others. A possible role of T-cells in causation of epilepsy is intriguing because it opens a whole suite of T-cell targeted treatments, including antigen-specific depletion of T-cells by CAR (chimeric antigen receptor) T-cells.[7]

In summary, the study by Kumar et al provides a detailed account of immune-related cellular interactions and signaling pathways in human patients with drug-resistant focal epilepsy. The ideas that microglia and T-cells may be critically involved in the pathogenesis of epilepsy, and that the disease shares similarities with multiple sclerosis, create unique therapeutic opportunities that can be tested in future clinical trials. However, the present investigation only included 6 pediatric patients with lesion-related epilepsies, and studies of larger patient cohorts will be necessary to validate the findings across different epilepsies and patient populations. Additionally, as expected from human tissue studies, the results are correlative and do not provide proof of causality. For example, it is unclear whether the immune perturbations are a cause or a consequence of the epilepsy. While some immunological conditions, such as Rasmussen's encephalitis can cause epilepsy, it has also been suggested from animal studies that seizures can cause or exacerbate brain inflammation. Finally, all patients included in the study suffered from intractable seizures despite treatment with 3 to 7 different anti-seizure drugs at the time of surgery. Thus, it is possible that the inflammatory changes were related to the use of anti-seizure drugs and mechanisms of intractability, rather than the epileptogenic process.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Varadkar S, Bien CG, Kruse CA, et al. Rasmussen's encephalitis: clinical features, pathobiology, and treatment advances. *Lancet Neurol*. 2014;13(2):195-205.
2. Bhatia S, Schmitt SE. Treating immune-related epilepsy. *Curr Neurol Neurosci Rep*. 2018;18(3):10.
3. Auvin S, Cilio MR, Vezzani A. Current understanding and neurobiology of epileptic encephalopathies. *Neurobiol Dis*. 2016;92(Pt A):72-89.
4. Aronica E, Crino PB. Inflammation in epilepsy: clinical observations. *Epilepsia*. 2011;52(suppl 3):26-32.
5. Kumar P, Lim A, Hazirah SN, et al. Single-cell transcriptomics and surface epitope detection in human brain epileptic lesions identifies pro-inflammatory signaling. *Nat Neurosci*. 2022;25(7):956-966. doi:10.1038/s41593-022-01095-5
6. Weltha L, Reemmer J, Boison D. The role of adenosine in epilepsy. *Brain Res Bull*. 2019;151:46-54.
7. Yi J, Miller AT, Archambault AS, et al. Antigen-specific depletion of CD4(+) T cells by CAR T cells reveals distinct roles of higher- and lower-affinity TCRs during autoimmunity. *Sci Immunol*. 2022;7(76):eabo0777.