Blood and cerebrospinal fluid immune cell profiles in patients with temporal lobe epilepsy of different etiologies

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INTRODUCTION

There is accumulating evidence for a role of the innate and adaptive immune system in epilepsy and epileptogenesis. Previous studies observed infiltration of myeloid cells and T lymphocytes in brain tissue of patients undergoing epilepsy surgery for various etiologies. Immune-mediated pathogenesis has been discussed in hippocampal sclerosis (HS) in particular. As brain specimens are gathered almost

Abstract

Inflammation plays a role in the pathogenesis of immune-mediated epilepsy, but also in epilepsy of other etiology such as hippocampal sclerosis. This study aimed to characterize immune cell signatures in the peripheral blood (PB) and cerebrospinal fluid (CSF) in temporal lobe epilepsy (TLE) of different etiologies. We retrospectively evaluated CSF routine parameters and immune cell profiles using flow cytometry in a cohort of 51 patients and 45 age-matched controls with functional disorders. Groups were comprised of patients with nonlesional TLE (n = 26), TLE due to hippocampal sclerosis (n = 14), or limbic encephalitis with antibodies against the 65-kDa isoform of glutamic acid decarboxylase (GAD65-LE; n = 11). TLE patients showed increased proportions of human leukocyte antigen–DR isotype (HLA-DR)-expressing CD4+ T lymphocytes in the CSF. Furthermore, they were characterized by a shift in monocyte subsets toward immature CD14lowCD16+ cells in the PB and blood/CSF-barrier dysfunction. Whereas TLE patients in general showed similar immune cell profiles, patients with GAD65-LE differed from other TLE patients by increased proportions of HLA-DR–expressing CD8+ T lymphocytes and type 2/3 oligoclonal bands. These findings point to a role of innate and adaptive immunity in TLE. CSF parameters may help to discriminate epilepsy patients from controls and different forms of TLE from each other.

KEYWORDS

autoimmune encephalitis, cerebrospinal fluid, flow cytometry, hippocampal sclerosis, immune signature, limbic encephalitis
exclusively during epilepsy surgery, their availability is limited. Blood and cerebrospinal fluid (CSF) provide more easily accessible specimens for studying the role of the immune system. Epilepsies due to autoimmune or limbic encephalitis (LE) have been identified as epilepsies with distinct immune-mediated etiology. A recent study in 68 LE patients showed an elevated blood CD4+CD8+ T-cell ratio compared to a small control group of temporal lobe epilepsy (TLE) patients. Studies on immune cells in the CSF in patients with other well-characterized epilepsy syndromes are lacking.

This study aimed to clarify whether patients with TLE exhibit specific immune cell alterations in the blood and especially CSF. We aimed to investigate whether TLE of different etiologies (LE, HS, and nonlesional TLE) share immunological features. A further goal was to identify factors assisting in differential diagnosis, as the distinction of LE from TLE of other etiology often poses a clinical challenge with major therapeutic consequences.

2 | MATERIALS AND METHODS

We retrospectively evaluated CSF, peripheral blood (PB) samples, and clinical history of 51 patients with epilepsy (Table S1). Cases were identified by screening the medical records from November 2013 to December 2018 for epilepsy patients who had received PB and CSF flow cytometry analysis. The detailed screening strategy is outlined in Methods S1. The study was approved by the local ethics committee. All patients eligible for inclusion were assigned to the following groups: TLE due to LE with autoantibodies against the 65-kDa isoform of glutamic acid decarboxylase (GAD65-LE; n = 11), TLE due to HS (TLE-HS; n = 14), TLE of unknown etiology (nonlesional TLE [nITLE]; n = 26). Age-matched patients who underwent CSF sampling due to functional disorders manifesting in neurological symptoms served as controls (n = 45; Table S1). All CSF samples were taken for clinical reasons such as exclusion or differential diagnosis of (meningo-)encephalitis. Flow cytometry is a routine part of CSF examination in our department and was performed as described elsewhere.6 Lynhocyte and monocyte subset frequencies were calculated as percentage of total lymphocytes and monocytes, respectively. Details on statistical methods are outlined in Methods S1.

3 | RESULTS

Demographic and clinical data as well as routine CSF parameters are shown in Table S1. Groups did not differ significantly in age, sex, or disease duration. Values for parameters identified in this study are provided on the patient level in Figure S1.

Analysis of conventional CSF data revealed an increased frequency of blood/CSF-barrier dysfunction in patients with TLE (Figure 1A,B). Flow cytometry revealed distinct immune cell profiles in the PB and CSF of patients with TLE compared to controls (Figure 1A,B). Immune cell parameters commonly differentiating TLE patients from controls in PB were a shift toward CD14lowCD16+ cells within monocytes and increased proportions of granulocytes (Figure 1A,B heatmap cluster 8), whereas proportions of lymphocytes and especially CD8+ T lymphocytes were decreased (Figure 1A,B heatmap cluster 2). In the CSF, TLE patients were characterized by reduced proportions of CD14lowCD16+ monocytes and increased proportions of human leukocyte antigen–DR isotype (HLA-DR)-expressing activated CD4+ T lymphocytes (Figure 1A,B heatmap cluster 2 and 8). In contrast to immune cell parameters commonly altered in TLE, significant alteration in other parameters was mainly driven by TLE subgroups (Figure 1B heatmap cluster 1 and 3-7). Proportions of B lymphocytes did not differ from controls in any of the TLE subgroups or the total TLE group.

Comparison of flow-cytometric immune cell parameters from the PB and CSF as well as conventional CSF parameters within TLE subgroups was performed to identify parameters assisting differential diagnosis. GAD65-LE patients differed from TLE-HS and nITLE by increased frequencies of oligoclonal band (OCB) type 2/3-positive patients (only seen in GAD65-LE patients) and elevated proportions of CD8+ HLA-DR–expressing CD4+ T lymphocytes (Figure 2A). Of note, these parameters also differentiated GAD65-LE patients from controls (Figure 1B box 5). After multiple group comparison with post hoc testing, the differences among TLE subgroups did not reach statistical significance. We constructed a composite score by multiplication of OCB positivity (1 for type 1 or 4, 2 for type 2 or 3) with proportions of HLA-DR–expressing CD8+ T lymphocytes of total lymphocytes (Figure 2B). Receiver operating characteristic analysis was used to define the cutoff best differentiating GAD65-LE from other TLE (Figure 2B right). The resulting cutoff of 9.9 separated the groups with a highly significant odds ratio of 15.1, providing a sensitivity of 85% and a specificity of 72.7%, resulting in an area under the curve of 85.7% (Figure 2B).

To exclude an impact of clinical variables (ie, disease duration and time since last seizure) on the investigated parameters, we correlated disease duration and time since last seizure (n = 35 patients with known exact date) with both conventional and flow cytometry parameters. Median latency of last seizure to CSF sampling was 8 days (mean = 45.5 days, standard deviation = 71.4). We found a weak negative correlation of time since last seizure and CSF leucocyte count (Spearman two-sided P = .025, r = -.379) and CD56bright (P = .026, r = -.375) and no correlation with disease duration.
In summary, we identified common shifts in monocyte and lymphocyte subsets in the PB and CSF including increased proportions of activated HLA-DR–expressing CD4+ T cells in the CSF as typical immunological features in TLE patients. Furthermore, GAD65-LE patients may be differentiated from other TLE patients by increased intrathecal CD8+ HLA-DR–expressing T-lymphocyte levels and IgG synthesis.

The most robust parameters that differentiated epilepsy patients from controls in our study were changes in the blood, and not CSF immune signatures. Our study cannot differentiate whether this is due to a primary systemic inflammatory response or a circumvention of the CSF compartment and more pronounced involvement of the small blood vessels and brain parenchyma. Lymphocyte clusters in the perivascular regions in resections from TLE patients may serve to support the hypothesis of CSF circumvention. Other immune parameters such as cytokines may show time-dependent increase or decrease after single seizures in TLE. The weak negative correlation of CSF leukocyte count and interval since last seizure in our study is in line with previous work. Apart from that, the lack of correlation of immune signatures to disease duration or the interval since last seizure may argue for a persistent shift in immune cell signatures and against a short-lasting seizure-related systemic inflammatory response. Thus, the immune signature of TLE seems to manifest early in the disease course.

**FIGURE 1** Distinct immune signature of epilepsy patients. A, Representation of the median (continuous parameters) or mean (categorical parameters) fold change and P values in parameters between controls (Ctrl) with functional disease (n = 45) and patients with epilepsy (nonlesional temporal lobe epilepsy [nTLE], n = 26; temporal lobe epilepsy due to hippocampal sclerosis [TLE-HS], n = 14; limbic encephalitis with antibodies against the 65-kDa isoform of glutamic acid decarboxylase [GAD65-LE], n = 11) in the peripheral blood (PB; left) and cerebrospinal fluid (CSF; right). Only significantly (P < .05) altered parameters are labeled. B, Heatmap visualizing the relative difference in the investigated parameters between the groups using hierarchical clustering (heatmapcluster 1-8). Red indicates the highest expression, whereas blue indicates the lowest expression. Parameter labeling provides information on the respective compartment (red, PB; blue, CSF). dysf., dysfunction; HLA-DR, human leukocyte antigen–DR isotype; OCB, oligoclonal bands.
With regard to the innate immune system, all TLE subgroups in this study showed a shift toward immature monocytes in the blood. In contrast, other studies showed an activation of classical monocytes or an increase of the frequencies of all monocytes. Animal models of status epilepticus yielded evidence for a pathogenic role of infiltrating monocytes in contrast to a mere epiphenomenon. Taken together, these results indicate an involvement of the innate immune system in TLE of different etiologies. A pathogenic role of innate immunity is also discussed for autoimmune encephalitis with monocyte infiltration as an initial step resulting in secondary activation of the adaptive immune system and development of encephalitis.

Concerning the adaptive immune system, an increase of activated CD4+ T lymphocytes in the CSF was a shared feature of the TLE patients, but most pronounced in TLE-HS patients. Histopathological studies in TLE-HS have shown the presence of T lymphocytes in hippocampal parenchyma, mostly in the perivascular region. There is also evidence for a correlation of the number of CD8+ T lymphocytes in the hippocampus with the degree of neuronal loss. In GAD65-LE, an increase of CD4+ T lymphocytes and their activated states was found to be more pronounced in the PB than the CSF, similarly to the finding of an elevated blood CD4+/CD8+ ratio in LE patients compared to controls in a recent study. On the other hand, in our study an elevated percentage of activated CSF CD8+ T lymphocytes helped to differentiate GAD65-LE from controls and...
other TLE. Histopathological studies have shown brain parenchyma infiltration of T lymphocytes and, more specifically, CD8+ T lymphocytes in GAD65-LE. The elevated proportion of activated CD8+ T lymphocytes in the CSF in our study may serve as additional evidence of a role of CD8+ cytotoxic T lymphocytes in the pathogenesis of GAD65-LE. In accordance with previous data, we found a high percentage of CSF-specific OCBs (45.5%) in GAD65-LE patients, indicating an intrathecal IgG synthesis, although B lymphocytes and plasma cells did not differ. Intrathecal IgG synthesis was not seen in patients with TLE-HS or nTLE.

The findings of this study concerning GAD65-LE patients may not be entirely representative for LE with other specific antibodies. Routine CSF parameters are known to show changes of cell or protein count and OCBs in varying degrees in LE. Because the immune response may follow different pathways depending on the specific antibody, we opted for a homogenous group of LE patients with GAD65-LE at the expense of group size rather than choosing a larger group of patients with multiple different antibodies.

Can flow cytometry be useful in distinguishing different TLE etiologies? TLE-HS and nTLE showed similar immune signatures. Whereas HS is usually reliably detected by magnetic resonance imaging (MRI), the distinction of TLE due to LE from other etiologies can be quite challenging, especially in the absence of a specific antibody or in the case of inconclusive MRI findings. We calculated a composite score aimed at distinguishing GAD65-LE from nTLE or TLE-HS in an attempt to differentiate immune-mediated from non–immune-mediated TLE. Future studies with higher numbers of LE patients with different antibodies and also seronegative LE patients may allow calculation of a similar score to serve that clinically highly relevant purpose. In addition, seronegative LE is likely to share some features of other limbic encephalitides, but diagnosis remains challenging, with an urgent need of reliable biomarkers, so studies focusing on this subtype are also needed.

5 CONCLUSION

This study provides further evidence of an involvement of the innate and adaptive immune system in TLE of different etiologies. It remains to be ascertained whether these findings are indicative of a causal role of the immune system in epilepsy or represent a state resulting from seizure activity. Our study shows that flow cytometry helps to differentiate between GAD65-LE and non–immune-mediated TLE and as such provides evidence that immune-mediated and non–immune-mediated TLE differ in their immunological profile.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS
S.K. and L.L. conceived the study and defined the concept. T.B. performed the database query. L.B., L.L., and S.K. screened the clinic letters and together with A.S.-M. analyzed the data. L.L. wrote the initial draft of the manuscript. A.S.-M. prepared the figures. B.S. reevaluated imaging data. S.K. extensively revised the manuscript for intellectual content. S.G.M., H.W., N.M., C.C.G., and C.E.E. advised on the study concept and interpreted some of the data. All authors contributed to the concept of the work and writing the manuscript, critically discussed the data, and approved the version to be published.

ETHICAL PUBLICATION STATEMENT
We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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