Brain, Behavior, and Immunity 53 (2016) 123–130

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Peripheral leukocyte profile in people with temporal lobe epilepsy reflects the associated proinflammatory state

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ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 25 November 2015
Accepted 26 November 2015
Available online 27 November 2015

Keywords:
Human temporal lobe epilepsy
Immunophenotyping
Lymphocytes
Cell activation
Cytokines

ABSTRACT

Introduction: Markers of low-grade peripheral inflammation have been reported amongst people with epilepsy. The mechanisms underlying this phenomenon are unknown. We attempted to characterize peripheral immune cells and their activation status in people with temporal lobe epilepsy (TLE) and healthy controls.

Methods and results: Twenty people with TLE and 19 controls were recruited, and peripheral blood lymphocyte and monocyte subsets evaluated *ex vivo* by multi-color flow cytometry. People with TLE had higher expression of HLA-DR, CD69, CTLA-4, CD25, IL-23R, IFN-γ, TNF and IL-17 in $CD4^+$ lymphocytes than controls. Granzyme A, CTLA-4, IL-23R and IL-17 expression was also elevated in $CD8^+$ T cells from people with TLE. Frequency of HLA-DR in $CD19^+$ B cells and regulatory T cells $CD4^+CD25^+Foxp3^+$ producing IL-10 was higher in TLE when compared with controls. A negative correlation between $CD4^+$ expressing co-stimulatory molecules (CD69, CD25 and CTLA-4) with age at onset of seizures was found. The frequency of $CD4^+CD25^+Foxp3^+$ cells was also positively correlated with age at onset of seizures.

Conclusion: Immune cells of people with TLE show an activation profile, mainly in effector T cells, in line with the low-grade peripheral inflammation.

1. Introduction

Epilepsy is a major public health problem affecting around 1% of the population worldwide. Temporal lobe epilepsy (TLE) is the most common epileptic syndrome in adults, and is frequently associated with difficult control seizures. Its etiopathogenesis is complex, involving both genetic and environmental factors. Taking into account their role in plastic or structural changes in several organs, experimental studies have focused on the involvement of inflammatory mechanisms in epilepsy (Silveira et al., 2012; Dedeurwaerdere et al., 2012; Vezzani, 2005; Vezzani and Granata, 2005).

Inflammation has generally been regarded as harmful to the brain as local (microglia) and infiltrating immune cells ($CD4^+$ and $CD8^+$ T cells), as well as pro-inflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF), and IL-6, may exacerbate neuronal damage in epilepsy (Vezzani, 2005; Liimatainen et al., 2013). Conversely, there is also evidence supporting a protective role for innate and adaptive immune cells as they can contribute to seizure-suppression in animal models of TLE (Ravizza et al., 2005; Zattoni et al., 2011). Whatever its effect or involvement, a growing body of evidence has supported a role for inflammatory mechanisms in epilepsy. This is mainly supported by the finding of high levels of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF, in the cerebrospinal fluid (CSF) or blood

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http://dx.doi.org/10.1016/j.bbi.2015.11.016
0889-1591/

of people with epilepsy, notably TLE (Li et al., 2011; Mao et al., 2013). The meaning and/or the triggers of this low-grade inflammation in epilepsy is unknown. Only a few studies have characterized the peripheral immune cells in TLE (Zattoni et al., 2011; Bauer et al., 2012; Deprez et al., 2011; Noe et al., 2013; Pitkanen and Lukasiuk, 2009, 2011; Ueda et al., 2013). For instance, one study reported elevated frequency of monocytes and NK cells in people with refractory epilepsy to antiepileptic drugs (AEDs) Nowak et al., 2011.

As previous studies reported increased levels of circulating pro-inflammatory cytokines and/or mediators, suggesting a persistent peripheral low-grade inflammation in TLE, we hypothesized that cells of people with epilepsy would display an activated profile when compared with controls. To test this hypothesis, we evaluated the activation status and the production of cytokines by peripheral immune cells.

2. Methods

2.1. Subjects

This study enrolled 20 people with TLE and 19 controls recruited from a tertiary referral center. The inclusion criteria for TLE were: diagnosis of TLE according to the ILAE criteria, age of more than 18 years, capacity to provide written informed consent, and seizure-free for at least 72 h reported by the people with TLE and/or their companion. This period was chosen as previous studies showed that circulating cytokines returned to basal levels at least 24 h after seizures (Nowak et al., 2011; Bauer et al., 2009). All patients had temporal mesial sclerosis on magnetic resonance imaging (MRI). Control group was recruited in Belo Horizonte, according to the following criteria: age more than 18 years and lack of any psychiatric disorder, any severe medical condition or neurological diseases including epilepsy. For both groups, the exclusion criteria were history of previous neurosurgery, use of anti-inflammatory and antibiotic drugs in the last two weeks, or cognitive impairment according to the Mini Mental State Examination and had other medical or neurologic diseases other than epilepsy. Socio demographic (age, gender, ethnicity, marital status, occupational status and educational level) and clinical data (weight and height, age of onset, duration of epilepsy, seizure type, seizure frequency, medication use, AED regimen, MRI and EEG findings) were also collected for both groups. This study was approved by local ethics research committee under the protocol number 607.264-0.

2.2. Peripheral blood mononuclear cell isolation and cell surface staining

Blood was collected after clinical interview and immediately processed. Whole blood cells were obtained from K3-EDTA venous vacuum tubes. Erythrocytes were lysed using ACK lysing solution (0.15 M NH_4Cl, 1 mM $KHCO_3$ and 0.1 mM Na_2EDTA) and washed twice with cold phosphate buffer saline (PBS) (1200 rpm, 4°, 10 min). After erythrocyte lysis, white blood cell (WBC) were stained with a combination of fluorescein isothiocyanate (FITC), phycoerythrin (PE), cy5.5-chrome (Cy)-labeled or PerCP 5.5, allophycocyanin (APC), cy7-allophycocyanin (APC) and cy7-phycoerythrin (PE-Cy7) antibodies directed against the surface molecules anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-CTLA-4, anti-CD69, anti-IL23R, anti-CD56, anti-CD19, anti-HLA-DR, anti-CD14, anti-CD16 and anti-CD86 (eBioscience, San Diego, CA, USA; BDPharMingen, San Diego, CA, USA and Invitrogen/Molecular Probes, Camarillo, CA, USA) for 20 min at 4 °C and data acquired using a FACSCantoII (Becton & Dickinson, San Jose, CA, USA).

2.3. Intracellular cytokines and FoxP3 staining

White blood cells were analyzed for their surface profile and intracellular cytokine expression pattern. Briefly, cells were fixed with phosphate buffer saline (PBS) and formaldehyde (2%) (Sigma–Aldrich, St. Louis, MO, USA) for 20 min. Fixed cells were permeabilized using saponin 0.5% (Sigma–Aldrich) and stained using monoclonal antibodies for granzyme-A, FoxP3, CTLA-4, TNF, IFN-γ, IL-17A and IL-10 (Invitrogen/Molecular Probes and BDPharmingen) conjugated with phycoerythrin (PE) or allophycocyanin (APC). PE and APC-labeled immunoglobulin control antibodies and a control of unstaining WBC were also included in all experiments. Preparations were acquired in FACSCantoII (Becton & Dickinson, San Jose, CA, USA). A minimum of 50,000 gated events in lymphocyte population was acquired for analysis due to the low frequency of positive events being analyzed.

2.4. Flow cytometry data analysis

Natural killer, T and B lymphocytes were analyzed for their intracellular cytokine and costimulatory surface marker expression pattern and frequency using the FlowJo program (Tree Star, Ashland, OR, USA). Limits for the quadrant markers were always set based on negative populations and isotype controls. At least three different fluorochromes were combined for each analysis. Gating strategy is depicted in Fig. 1. After gating specific cell subsets,

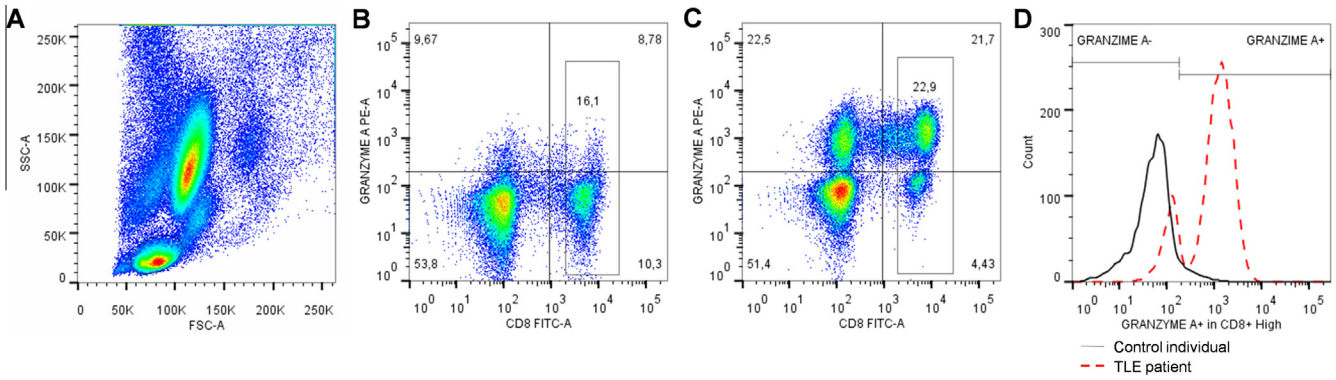

Fig. 1. Representative flow cytometry graphs of $CD8^+$ T cells expressing granzyme A from control individual (CT) (B) and participant with temporal lobe epilepsy (TLE) (C). Flow cytometry dot-plots demonstrate the region of total lymphocytes (A) and the data analyzed in $CD8^+$ T cells (B and C) expressing granzyme A and histograms (D) in controls (black line) and TLE (red dashed line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).