Mass Cytometry Immune Cell Profiling in an Experimental Mouse Model of Epilepsy-Associated Focal Cortical Dysplasia (FCD)

Poster 3.073

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Background

Focal cortical dysplasia (FCD) is a group of localized cortical malformations responsible for focal epileptic seizures, which are highly refractory to current antiseizure medications (ASMs).[1,2] Several publications have identified that an increased proportion of patients with FCD have mutations in the mTOR pathway, a crucial regulator of cell growth and survival.[1,2] Many of these mutations are somatic and result in mosaicism in the brain, which has led to difficulties in understanding the functional consequences of these mutations. Therefore, robust experimental models of epilepsy-associated FCD are needed to develop more targeted and potential disease modifying treatments.[3]

Objective

The goal of the present study was to extend the characterization of a developmental mouse model of epilepsy-associated FCD with a group housing video-EEG wireless telemetry system. Concurrently, using CyTOF mass cytometry, immune cell profiling was investigated, assessing central and peripheral immune activation as well as infiltration in brain and spleen.

Methods

- We have evaluated a mouse model of epilepsy-associated FCD using *in utero* electroporation of mutations (Rheb mutated S16H plasmid) into the embryonic brain[3,4]. In order to assess behavioral phenotypes in this model, we implemented a group housing video-EEG wireless telemetry platform that provides continuous recording of video-EEG 24 hours/day without supervision. Data was collected from cohabiting controls and FCD freely moving mice.
- For immune cell profiling characterization, CyTOF mass cytometry[5] was employed, a high dimensional analog of flow cytometry involving mass spectrometry-based analyses of single cells bound with metal-tagged antibodies.

All experiments involving mice were conducted in compliance with guidelines issued by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the ethical committee for animal experimentation according to Belgian law and the European Committee Council directive (2010/63/EU).

Overview

QUESTION

There is a clear need to improve our understanding of the etiology of FCD and epileptogenesis to identify novel therapeutic treatments. Is there a role for inflammatory responses in the development of the FCD phenotype?

INVESTIGATION

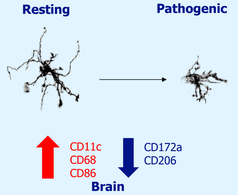
Extend the characterization of a developmental mouse model of epilepsy-associated FCD with a high throughput group housing video-EEG wireless telemetry system and investigate the immune cell profiling by CyTOF mass cytometry.

RESULTS

Experimental FCD model

Results

- The model recapitulates human pathobiology of FCD.
- Cortical malformations.
- Spontaneous recurrent seizures associated with isolated behavior and reduced social interaction.
- Microglia, neutrophil and dendritic cells phagocytic activation markers were increased in brain (CD68, CD86, CD11c) and spleen (CD68 and CD44).
- Microglia appeared highly phagocytic and activated in the lesional area (ipsilateral) in chronic stages of FCD animal model.



CONCLUSIONS

Microglial activation might play an important role in the pathogenesis of epilepsy-associated FCD by maintaining chronic inflammatory responses. There is a clear need to investigate these mechanisms in epilepsy and FCD to develop novel and more efficacious drug therapies for the treatment of FCD patients.

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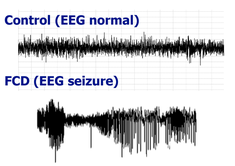
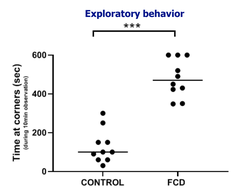
Group housing video-EEG wireless telemetry recordings

- Group housing telemetry system enables high-throughput studies with mouse models of epilepsy with unparalleled quality video-EEG recordings in real time of grouped-housed animals and high sampling over extended periods of time.
- It allows to define the seizure-onset zone and record up to 160 animals (group housed) at the same time (24/7 without supervision). Data can be collected from cohabiting animals exercising free behavior.
- These recordings allows us to identify specific behavioral patterns and EEG abnormalities in the model.



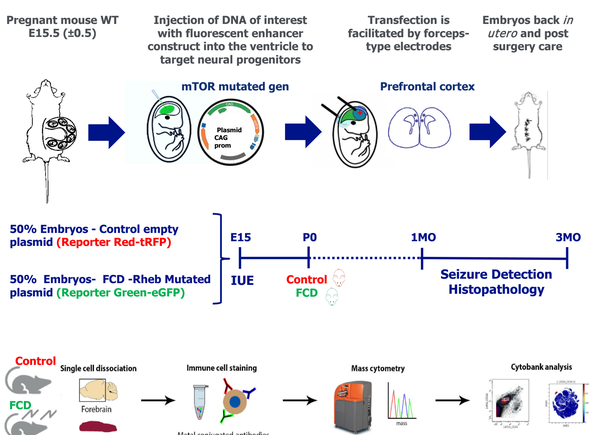


- FCDs generated in prefrontal cortex caused by mTOR hyperactivity led to spontaneous and recurrent electrographic and tonic-clonic seizures. 85% of mice expressing the Rheb mutated protein display electrographic seizures (8-10 seizures/day, 30-60 s duration) with convulsive behavior.
- Exploratory behavior analysis showed that FCD mice with recurrent seizures exhibit several symptoms of isolated–autistic like behavior, including reduced social interactions spending significantly less time exploring the cage compared to control littermates.

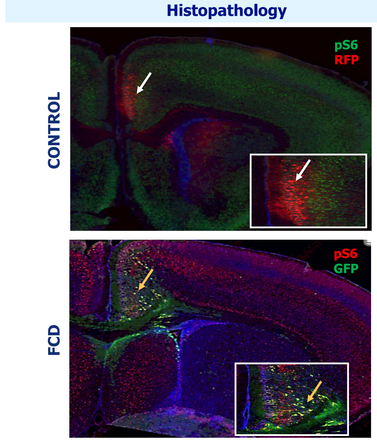
Conclusions

- The model recapitulates the human pathobiology of FCD with manifestation of spontaneous and recurrent seizures that closely resemble the dysplastic histopathologies. Microglia appears highly phagocytic and activated and might play an important role in the pathogenesis of epilepsy-associated FCD by maintaining a chronic inflammatory responses.
- The group housing video-EEG telemetry platform allows us to investigate the presence of distinctive social behavioral phenotypes to evaluate new drug treatments on animal behavior and establish a link between cortical malformations, seizures and neurodevelopmental comorbidities.

In utero electroporation



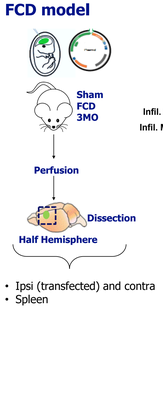
Histopathology



RFP (red-fluorescent protein) is expressed in control mice highlighting the normal cortical organization (white arrow). FCDs generated in the prefrontal cortex caused by mTOR activity upregulation (pS6 staining in red and green-fluorescent protein in green) led to focal cortical disorganization (yellow arrow). The animal model accurately reproduces the FCD pathobiology with a clear disrupted neural migration, cortical dyslamination and dysplastic neurons.

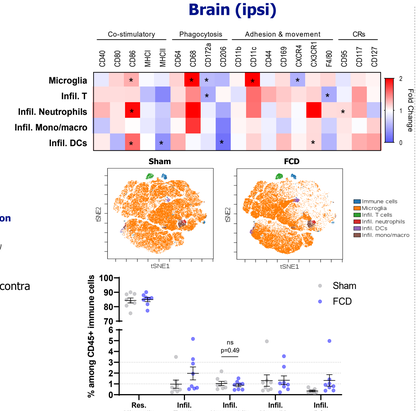
CyTOF profiling

FCD model

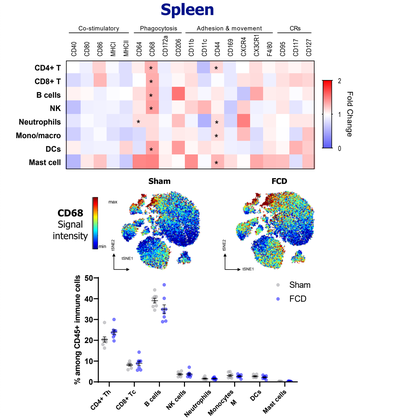


- Ipsi (transfected) and contra
- Spleen

Brain (ipsi)



Spleen



No significant changes in the percentage of resident or infiltrating cell populations in ipsilateral brain but a trend for increased dendritic cells (DC) and T cell infiltration in FCD cases was observed. The functional analysis showed significant increased expression of activation markers (CD68, CD11c, CD86) and significant decreased expression of 'don't-eat-me' (CD172a) and anti-inflammatory (CD206) markers in FCD ipsilateral brain, particularly in microglia. Activation significantly increased in spleen, particularly CD68 phagocytic marker.

References

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