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Intracerebral delivery of the M2 polarising cytokine interleukin 13 using mesenchymal stem cell implants in a model of temporal lobe epilepsy in mice

Idrish Ali¹, Stephanie Aertgeerts¹, Debbie Le Blon², Daniele Bertoglio¹, Chloe Hoornaert², Peter Ponsaerts², Stefanie Dedeurwaerdere¹

1. Department of Translational Neurosciences, University of Antwerp, Belgium

2. Laboratory of Experimental Hematology, Vaxinfectio, University of Antwerp, Belgium

Corresponding author:

Dr. Idrish Ali

Department of Translational Neurosciences, University of Antwerp, FGEM CDE T4.98, Universiteitsplein 1, Wilrijk, Antwerp 2610, Belgium

Phone number: +32-3-2652638

idrish.ali@uantwerpen.be

Running title: M2 polarising cytokine in epilepsy

Key points:

- IL13 producing mesenchymal stem cells (IL13 MSC) implants neither affect status epilepticus (SE) nor chronic epileptic seizures
- Robust IL13 MSC induced M2 polarisation of microglia/macrophages at the MSC graft site in the hippocampus, but not in the lesion site

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- M2 polarised microglia/macrophages at the MSC graft site display a strong expression of translocator protein

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Abstract

Objectives: Neuroinflammation plays a critical role in the pathophysiology of mesial temporal lobe epilepsy. We aimed to evaluate whether intracerebral transplantation of Interleukin 13 producing mesenchymal stem cells (IL13 MSC) induces an M2 microglia/macrophage activation phenotype in the hippocampus with an epileptogenic insult, thereby providing a neuroprotective environment with reduced epileptogenesis. **Methods:** Genetically engineered syngeneic IL13 MSC or vehicle was injected within the hippocampus one week before the intra-hippocampal kainic acid-induced status epilepticus (SE) in C57BL/6J mice. Neuroinflammation was evaluated at disease onset as well as during chronic epilepsy period (9 week). In addition, continuous vEEG monitoring was obtained during the chronic epilepsy period (between 6-9 weeks after SE). **Results:** Evaluation of vEEG recordings suggested that IL13 MSC grafts did not affect the severity and duration of SE or the seizure burden during the chronic epilepsy period, when compared to the vehicle treated SE mice. An M2 activation phenotype was induced in microglia/macrophages that infiltrated the IL13 MSC graft site, as evidenced by the arginase1 expression at the graft site at both 2-week and 9-week time point. However, M2 activated immune cells were rarely observed outside the graft site and accordingly, the neuroinflammatory response or cell loss related to SE induction was not altered by IL13 MSC grafting. Moreover, an increase in the proportion of F4/80⁺ cells was observed in the IL13 MSC group compared to the controls. **Significance:** Our data suggest that MSC based IL13 delivery to induce M2 glial activation does not provide any neuroprotective or disease modifying effects in a mouse model of epilepsy. Moreover, use of cell grafting to deliver bioactive compounds for modulating

neuroinflammation may have confounding effects in disease pathology of epilepsy due to additional immune response generated by the grafted cells.

Introduction

Temporal Lobe Epilepsy (TLE) is the most common form of focal acquired epilepsy in adults. Around one-third of the patients remain drug resistant and the current available treatments are only symptomatic, which emphasizes a strong need for disease modifying and possible preventive therapies¹. Over the past decade, a plethora of evidence has accumulated from human tissue and animal models, supporting an important role of brain inflammation in epilepsy. A pro-inflammatory response can contribute to the progression of overall network excitability^{2, 3} and seizure-induced cell death^{4, 5}, affect survival of seizure-induced new adult born neurons and their expression of synaptic proteins⁶. Moreover, suppressing neuroinflammation to limit the epileptogenesis after a precipitating injury has been widely described⁷⁻¹⁰.

Brain inflammation is known to involve a broad spectrum of molecules and processes and this heterogeneity may limit the therapeutic benefits of a complete inhibition. A typical immune response after an injury leads to a classical M1 immune activation, which contributes to neuropathology by being highly pro-inflammatory and cytotoxic, but also enables the clearance of damaged tissue. On the other hand, M2 activation with anti-inflammatory markers promotes repair mechanisms¹¹. Indeed strategies promoting M2 activation have showed positive outcomes in several brain disorders¹²⁻¹⁴. A recent study by Benson et al. in 2015, showed a mixed expression of M1 and M2 activation markers in the chronic phase of epilepsy¹⁵. Importantly, the levels of M1 markers were significantly higher during the early periods after status epilepticus (SE), without any changes in the M2 markers compared to the control animals. Similarly, a shift of M1/M2 balance towards M1 was observed at epilepsy onset in a genetic model of epilepsy¹⁶. Thus, we hypothesised that modulating this M1/M2 balance towards M2 activation would limit the damaging effects of an epileptogenic insult.

Recent studies used intracerebral grafting of mesenchymal stem cells (MSC) for in situ delivery of the M2 polarising cytokine interleukin 13 (IL13). These studies found a reduced microglia/macrophage immune responses upon grafting IL13 MSC, during cuprizone-mediated CNS inflammation and demyelination¹⁷, as well as following spinal cord injury¹⁸. In these specific models, a major inflammatory component precedes primary or secondary cellular injury, and the induction of M2 alternative activation of microglia/macrophages may thus be directly linked to reduced downstream microglia/macrophage cytotoxicity. However, it remains to be investigated whether IL13 can alter

neuroinflammatory responses that are primarily initiated by excitotoxicity mediated by an epileptogenic brain injury and thereby may influence epileptogenesis. Thus, in this study we aimed to evaluate the effect of IL13 producing genetically modified mesenchymal stem cells on SE induction as well as development of epileptic seizures and associated neuropathological alterations in intra-hippocampal kainic acid (KA) induced mouse model of epilepsy.

Materials and Methods:

Animals

Eight-week-old (20-25 g) male C57Bl/6 (Charles River) were individually housed on a 12:12 light/dark cycle with food and water *ad libitum* under a temperature ($22 \pm 2^\circ\text{C}$) and humidity ($55 \pm 10\%$) controlled environment. All animals were acclimatized for at least five days before the start of the experiments and all procedures were performed according to the European Ethics Committee (decree 86/609/CEE) and the study protocol was approved (ECD 2014-39) by animal ethics committee of the University of Antwerp, Belgium.

Study design

A total of 67 mice were used for the study. For the 2-week histology study, eighteen mice were used of which 14 mice belonged to either of the treatment groups (vehicle; $n=7$ or IL13 MSC; $n=7$) that underwent status epilepticus (SE) and 4 mice to the naïve control group. For the 9-week time point, an additional 49 mice were used (for vEEG and histological evaluations) where 44 mice belonged to the treatment groups (MSC IL13; $n=21$ and vehicle; $n=23$) and 5 as naïve controls. A total of 7 mice died during SE or in the following weeks. Moreover, mice that lost their electrode assembly ($n=5$), experienced a SE duration of less than 3 hours ($n=2$ in vehicle and $n=1$ in IL13 MSC group) or presented artefacts in EEG signal ($n=2$) were excluded from the study. Mice were injected with IL13 MSC or vehicle one week before SE induction. Mice were injected with kainic acid to induce SE and were sacrificed at two time points (Figure 1). The animals in the 9-week cohort were also implanted with electrodes after kainic acid injections and underwent continuous vEEG recordings from 6 weeks until 9 weeks after SE inductions.

Genetic modifications of MSCs

In this study, we used a previously established and characterised C57BL/6 mouse bone marrow-derived MSC line, genetically engineered to co-express the blue fluorescent protein and interleukin-13

(further named as IL13 MSC)¹⁷. For expansion, IL13 MSC were cultured in standard cell culture flasks in 'complete expansion medium' (CEM) consisting of Iscove's modified Dulbecco's medium (IMDM; Lonza) supplemented with 8% fetal bovine serum (FBS; Invitrogen), 8% horse serum (HS; Invitrogen), 200U/ml + 100 µg/ml penicillin/streptomycin (Invitrogen), 1 µg/ml amphotericin B (Invitrogen) and 5 µg/ml puromycin (Invivogen). Expression of IL13 was confirmed by a murine IL13 ELISA, demonstrating a secretion of 76 ± 3 ng IL13/6x10⁴ cells/24 h. MSC cultures were split 1:5 twice a week using 0.05% trypsin-EDTA (Invitrogen) for cell detachment. For injection experiments, harvested cells were washed twice with PBS before re-suspending in PBS at the appropriate concentration (see below).

Cell implantation, SE induction and electrode implantation surgeries

All the surgeries were performed in sterile conditions, under deep isoflurane anaesthesia (5% for induction and 1.5% for maintenance). For the 2-week time point, IL13 MSC (20000 in 1.5 µl PBS) or vehicle was injected bilaterally in the ventral hippocampus at the following coordinates from the bregma: anteroposterior (AP) = -2.95, mediolateral (ML) = 3.0, dorsoventral (DV) = -2.35 mm, using an automatic microinjector pump (KD Scientific, Holliston, MA, USA) with a 10 µl 30-gauge Hamilton syringe (Hamilton, Bonaduz, Switzerland). For the 9-week time point, two injections were made in the dorsal hippocampus, at sites rostral and caudal to the kainic acid injection site [AP = -1.45, ML = 1.0, DV = 1.9 mm and AP = -2.7, ML = 2.3, DV = -1.65 mm], to maximize the effects in the brain hemisphere with the epileptic focus. The needle remained in place for 4 minutes to prevent backflow of the injected cell suspension. The skin was sutured and mice placed under a heating lamp to recover.

For SE induction mice were injected with kainic acid (50 nl of 20 mM) in the right hippocampus [AP = -2, ML = -1.4, DV = -1.8 mm] using a 32 G needle, Hamilton Neuros syringe (HA 65457-01) with the microinjector pump. Immediately after the kainic acid injection, a bipolar electrode (Plastics One Inc., USA) was implanted at the site of kainic acid injection and a third electrode was placed over the cerebellum as the ground. Electrodes and connector were fixed on the skull with dental acrylic cement¹⁹.

vEEG monitoring and seizure evaluations

After the SE induction, mice were connected for vEEG recording or video only (2-week time point), during 24-48 h and again between 6 weeks until 9 weeks after SE induction. Following the surgery, the animals immediately underwent SE. The animals were connected to the digital EEG acquisition system (Ponemah P3 Plus; Data Sciences International, USA) through a cable system with continuous video monitoring as previously described²⁰. EEG data was manually analysed by a researcher blind to the treatment using NeuroScore software (Data Science International, USA). For evaluation of SE, the duration and severity of the seizures was evaluated. The severity of SE was categorised using a score from 1 to 6 (supporting table 1) based on whether only non-convulsive seizures were observed or few/many events of convulsive seizures (class 3 to 5 based on a modified Racine's scale; supporting table 2) were observed during the period of SE²¹.

For chronic vEEG, seizures were categorized to either convulsive seizures (class 3-5) or hippocampal paroxysmal discharges (HPDs), which are events with at least twice the amplitude of the baseline recordings with a frequency of >8 Hz, as described previously in Riban et al²². Other pathological evaluations included seizure-like events (SLEs), which were low frequency high voltage sharp waves (usually <5 Hz) that were at least 5 s in durations as previously described by Twele et al²³. Most HPDs and SLEs were not associated with any behaviour. Though, subtle events like facial clonus and freezing may be missed in the vEEG recordings.

Tissue processing and Immunohistochemistry

Mice were perfused with 10ml phosphate buffer saline (PBS) and a 4% paraformaldehyde (PFA) solution in PBS. The brains were collected and post fixated in 4% PFA for another 2 hours. Next, the brains were treated with increasing concentrations of sucrose solutions (5% and 10% for 2 hours each) and 20% sucrose solution overnight. The brains were snap frozen in isopentane (at -50°C), and subsequently preserved in a -80°C freezer. For histological evaluations, serial coronal sections (10 µm) encompassing the hippocampus were cut on a cryostat, slide-mounted and stored at -80°C until further use. Sections were collected on Superfrost plus slides and distributed into 20 series so that each slide held every 20th section. For detailed methodology of immunohistochemistry, see supplementary methods.

Immunohistological quantification

Quantitative analysis for immunofluorescence images was performed using NIH ImageJ analysis software (v1.47) and TissueQuest immunofluorescence analysis software (TissueGnostics GmbH,

Vienna, Austria, v3.0), as previously described^{19, 24}. For Iba1⁺ cells and Iba1/Arg1 double positive cells the area of the hippocampus was delineated and the number of single positive and double positive cells were determined using the TissueQuest software. Similarly, the number of F4/80 positive cells in the hippocampus was quantified. The analysis was performed on dorsal hippocampus without IL13 cell grafts to avoid confounding effects of immune response due to the grafts. Similarly, to investigate cell loss and granule cell layer (GCL) dispersion within the hippocampus, the number of cells per unit area of the GCL was determined. There was very extensive cell loss observed in the pyramidal cell layers, and the cell loss scores (with a score of 4 for normal to 0 for total cell loss) were determined as previously described¹⁹. In addition, astrocytosis was evaluated by determining the percentage of GFAP⁺ area using ImageJ software¹⁹.

TSPO autoradiography

TSPO expression was detected by in vitro autoradiography using ³H-PK11195 (PerkinElmer, USA), a selective radiolabelled ligand of TSPO¹⁹. Briefly, brain sections were incubated in 50 mM Tris-HCl buffer containing 1 nM ³H-PK11195 or with additionally high concentration (20 µM) of non-labelled PK11195 (Sigma-Aldrich, USA) to determine total binding and non-specific binding, respectively. The sections were then exposed to a Hyperfilm, which was then developed and digitised as previously described¹⁹. The optical density (OD), measured from ImageJ software was converted to doses of radioactivity (Bq/mg tissue) with the ³H calibration values using GraphPad Prism6.

Statistical analysis

The Kolmogorov-Smirnov test showed that not all data sets were normally distributed and therefore it was decided to use non-parametric tests for the analyses. Mann-Whitney U test was performed to determine differences between veh-SE and IL13 MSC-SE animals for evaluations of SE and chronic seizure burden. The histology variables were compared between control, veh-SE and IL13 MSC-SE animals using a Kruskal-Wallis test with a *post-hoc* Dunn's multiple comparisons test. All analyses were done using GraphPad Prism 6, except for the Kruskal-Wallis test, which was done using SPSS (IBM SPSS Statistics 20, USA). Statistical significance was set at $p \leq 0.05$.

Results

Effects of IL13 producing cell grafts on seizure burden during chronic epilepsy

We compared the characteristics of SE between the mice that received vehicle or IL13 MSC grafts one week before SE induction. Control MSC grafts were not included, since we aimed for a proof of concept study investigating whether hippocampal pathology and epilepsy development mediated by epileptogenic insult could be altered by the production of IL13 within hippocampus. The severity scores were not significantly different between the veh-SE and IL13 MSC-SE mice (Figure 2A; $p=0.45$). Similarly, we observed no significant differences in terms of the duration of SE between the veh-SE and the IL13 MSC-SE mice (Figure 2B; $p=0.79$). Only animals with seizure duration of more than 3 h were included in the study. The animals experience mostly partial seizures with intermittent convulsive seizures, which were not significantly different between the groups (Figure 2C, $p=0.89$). During the chronic period, the vEEG were evaluated manually and categorized either as seizures with convulsive behavior (Figure 2D1) or hippocampal paroxysmal discharges (HPDs; figure 2D2). HPDs typically lasted for 20-25 s and may or may not be associated with behavioural phenotypes of non-convulsive partial seizures (Class 1-2, Racine's scale). However, the incidence of HPDs was rare (< 2 HPDs/day) unlike previously reported by Riban et al²². This was in line with the findings from²³, who also reported HPDs to be rare events. Accordingly, we observed a very high incidence (average of around 15/hour) of high amplitude low frequency (typically less than 5 Hz) spikes/sharp waves (Figure 2D3) and defined them as SLEs as was reported in Twele et al²³. While comparing between the veh-SE and IL13 MSC-SE mice, no significant differences in the convulsive seizures (Figure 2E; $p=0.89$), HPDs (Figure 2F; $p=0.08$) or the SLEs (Figure 2G; $p=0.68$) were observed. Similarly, no significant differences were observed between the treatment groups for the mean duration of convulsive seizures (Figure 2H; $p=0.95$), HPDs (Figure 2I; $p=0.99$) or SLEs (Figure 2J; $p=0.97$).

IL13 producing cell grafts did not affect neuroinflammation in the chronic epilepsy period

Histological evaluations were performed to confirm if IL13 production in the brain was able to induce a long-term M2 type microglia/macrophage activation phenotype within the hippocampus. At 10 weeks after cell grafting (9 weeks after SE), BFP expressing MSCs could be readily seen at the graft site (Figure 3A). As previously described¹⁷, the cell grafts were infiltrated with Iba1⁺ microglia/macrophages due to intrinsic immune recognition of syngeneic MSC implants (Figure 3B). MSC graft infiltrating immune cells were M2 polarised as evident by a strong expression of arginase1 ($>90\%$ of total cells) (Figure 3C-D). However, the arginase1⁺ cells were located only at the graft site and were rarely observed outside the graft site (0.6% and 1.4% of total Iba1+ cells for vehicle and IL13 MSC group respectively). Accordingly, no significant differences were observed between the IL13

MSC-SE and veh-SE animals for the density of Iba1⁺ cells, which were significantly higher in both groups when compared to the healthy controls (Figure 3E-H). To confirm these findings, we also evaluated expression of TSPO, a marker of neuroinflammation. Results from TSPO autoradiography showed no differences in the TSPO expression between the vehicle and IL13 MSC treatment group. In addition, we measured the density of F4/80⁺ cells that represent the activated microglia/macrophages (Figure 3I-K) within the hippocampus (thereby excluding the MSC graft site). The density of F4/80⁺ cells was significantly higher in the animals that underwent SE as compared to the healthy control animals. Furthermore, the density of F4/80⁺ cells in the hippocampus was also significantly higher in the IL13 MSC than in veh-SE group. The percentage of GFAP⁺ area was evaluated to determine any effects on the astrocyte activation. There were no significant differences between the veh-SE and IL13 MSC-SE animals, though the percentage of GFAP⁺ area was higher in both groups when compared to the healthy controls (Figure 3Q-T). Finally, we also evaluated whether IL13 producing cell graft provided protection against pathological neurodegeneration. We observed no changes in the GCL density or the pyramidal cell loss scores in the hippocampus while comparing the veh-SE and IL13 MSC-SE animals (Figure U-Y).

IL13 producing cell grafts did not affect neuroinflammation 2 weeks after SE induction

Because no obvious M2 polarisation of hippocampal inflammatory responses were observed at 9 weeks after SE, apart from microglia/macrophages within the MSC graft, we questioned whether or not this may have occurred at an earlier time point post SE induction. BFP expressing MSCs were seen abundantly at this time point (Figure 4A) and did not appear to migrate away from the injection site. At two-weeks post-grafting, MSC grafts were already infiltrated with Iba⁺ microglia/macrophages, which were forced to an M2 polarisation as evident by a strong expression of arginase1 within the graft site (Figure 4 B-D). Similar to 9-week time point, arginase1 expressing cells were rarely observed in the hippocampal areas away from the IL13 MSC graft (0.2% and 0.07% of total Iba1⁺ cells for vehicle and IL13 MSC group respectively). There was a strong increase in the Iba1⁺ cell density in the mice that underwent SE in both the dentate gyrus (including the molecular layer, GCL and dentate hilus) and pyramidal cell layers of hippocampus (Figure 4E-G). However, no significant differences were observed between veh-SE and IL13 MSC-SE groups (Figure 2H&I). We confirmed these findings by evaluating the TSPO autoradiographic images in these animals (Figure 3J-L). Though the specific binding of the TSPO was higher than the naive control animals (dotted line), no significant differences

were observed in the hippocampal TSPO expression between the vehicle and IL13 MSC injected groups (Figure 3M).

While evaluating the TSPO autoradiographs, it was also observed that the TSPO binding was denser at the sites of the cell grafts (Figure 3N). We aimed to confirm whether TSPO was present on the M2 polarised microglia/macrophages located within the IL13 MSC graft site. A double immunofluorescence staining of TSPO and Ym1 (marker for M2 activation) showed a strong TSPO expression in the cells at the graft site that were also Ym1 positive (Figure 3O-Q).

Discussion

We evaluated whether IL13 producing MSCs induces an alternative activation of microglia/macrophages in the hippocampus after SE and if such effects accompany a better outcome for seizure burden during the chronic epilepsy period. IL13 production by the grafted cells induced sustained M2 activation within the MSC graft infiltrating Iba1⁺ inflammatory cells, but not within the Iba1⁺ inflammatory cells present in the epileptic hippocampus. This suggests that the effects of IL13 release - in particular for this disease model - may not influence global neuropathological changes within the epileptic hippocampus. Accordingly, independent of the presence of Arg1⁺ cells within the MSC graft, IL13 MSC grafting did not translate into a better outcome in terms of the seizure burden or M2 conversion of hippocampal inflammatory response related to SE induction.

We performed vEEG monitoring from 6 weeks until 9 weeks after SE to evaluate the effects of IL13 producing cell implants on seizures. This time point was chosen in line with the reports that by this time the number of observed HPDs in this model is highest and remains stable there after²⁵. We observed a trend for a reduced number of HPDs in the mice with IL13 producing cell grafts compared to the control animals. However contrary to previous reports from Riban et al.²², the proportion of HPDs was very low in terms of total number of observed seizures, which was in line with previous reports²³. However, overall it can be concluded that IL13 production in the mice hippocampus did not alter the total seizure burden during chronic epilepsy period.

Since the vEEG recordings were performed only during the chronic period and not during the period of epileptogenesis, a possibility of a delay in the development of chronic seizures cannot be ruled out, though, clinical relevance of such an effect would be uncertain. Similarly, it needs to be taken into account while evaluating our data that the implantation of the cell grafts were performed before the induction of SE. This strategy was chosen for two main reasons: firstly, since the implanted cells are known to be attacked by glial cells²⁴, implanting the grafts after the SE induction would have been an

additional confounder for the measurement of the neuroinflammatory response. Further, this way it could also be ensured that a persistent and unvarying release of IL13 was available immediately after SE. Secondly, anti-inflammatory cytokines are also reported to modulate neuronal excitability via GABAergic transmission^{26, 27}. Hence it was also interesting to evaluate any anticonvulsive effects of IL13 producing cell grafts. We confirmed from our evaluations of SE that the IL13 cell implants did not alter any parameters measured during SE induction. A possible limitation of this study can be the lack of inclusion of a group of animals with control MSC grafts. However, the aim of the study was not to present effects of different MSC graft on neuroinflammatory responses but to provide a proof of concept study investigating whether hippocampal pathology mediated by epileptogenic insult can be altered by the local production of IL13 and whether that affected epilepsy development. Previous data from our work has already shown that control MSCs do not induce an M2 polarised activation state¹⁷. Moreover, there is a report that control MSC implantations do not improve seizure burden in epileptic rats²⁸.

The vEEG results were in line with the histological evaluations, where we observed that despite a strong M2 activation phenotype induced by IL13 within the graft, the microglia/macrophages or astrocytes in epileptic hippocampi away from the graft were not altered. Accordingly, no protection was observed for neurodegeneration within the epileptic hippocampus in mice with IL13 MSC grafts. To elucidate if there could be positive effects of hippocampal IL13 production at disease onset, we also performed microglial evaluations at 2-week after SE induction. This time point was chosen since it is around this time point the HPDs and high amplitude sharp waves are first detected²² and any inflammation related to seizures would be at minimum. Similar to the chronic period, no/minimal number of microglia/macrophages outside the MSC graft site expressed M2 markers. Accordingly, M2 activation within the MSC graft or IL13 produced by the grafted MSCs could not alter microglia/macrophage density within the hippocampus away from the graft.

There could be several reasons that may describe the lack of a neuroprotective or anti-inflammatory effects by the IL13 producing grafts. It is possible that the released IL13 from the cell grafts or any indirect contribution from the M2 polarised microglia/macrophages may not diffuse into the hippocampus due to a strong astrocytic scar that is reported to form around these grafts²⁴. However, recent studies have described possible release of IL13 outside IL13 MSC grafts by showing beneficial effects in mouse models of cuprizone-induced CNS demyelination and spinal cord injury. In these studies, the behaviour of pathology-associated microglia/macrophages was clearly altered (either being diminished or being M2 polarised), suggesting that IL13 may be involved in active CNS

immunomodulation subsequently conferring neuroprotection^{17, 18}. However, these potential mechanisms do not seem to be involved in this study, as no difference was observed between the hippocampal inflammatory responses in the control/vehicle and IL13 MSC groups. Alternatively, it is also possible that IL13 mediated effects are dependent on initial insult that leads to neuropathological events. An epileptogenic insult such as SE induces a strong acute inflammatory response due to excitotoxicity mediated neuronal death. This may be different from the neuroinflammatory responses induced by cuprizone-intoxication or following spinal cord injury. Especially for the latter, a clear natural M1 to M2 conversion is more apparent in the injured spinal cord, and may be enhanced by grafting IL13 MSC. In contrast to preceding studies, we here observed an increase in the number of F4/80 positive activated immune cells in the epileptic hippocampus. Therefore, it cannot be excluded that any protective effects from IL13 production may be counteracted by the immune response generated by the MSC grafts in hippocampus.

In an attempt to confirm the results from immunofluorescence staining we also performed TSPO autoradiography, which displayed a strong TSPO binding at the site cell grafts. TSPO is one of the most commonly used imaging biomarker of neuroinflammation and is believed to represent a pro-inflammatory response²⁹. However, our data suggests that even M2 activated microglia/macrophages may express high levels of TSPO, which we later confirmed by TSPO and Ym1 double immunofluorescence staining. Recent studies have reported that expression of TSPO may be driven to resolve inflammation³⁰ and that it is expressed on CD206 positive cells (marker of M2) in a mouse model of neuroinflammation induced by intracerebral hemorrhage³¹. At the same time, TSPO was also colocalised with CD16/32 positive cells (a marker of M1) in this model. Thus, it can be suggested that both M1 and M2 activated cells may express TSPO and that TSPO expression may not be solely a marker of a pro-inflammatory reaction in the brain.

In summary, our findings suggest that MSC mediated delivery of an M2 polarising cytokine may induce confounding effects in a mouse model of epilepsy due to increased inflammatory responses. In a recent study, viral vector based delivery of IL13 has also been shown to display protective effects against cuprizone-induced demyelination, associated with a more profound and uniform *in vivo* polarisation of microglia/macrophages towards an M2 activation phenotype¹⁴. However, it remains to be investigated whether this alternative approach would be effective in modulating inflammation after an epileptogenic insult.

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Disclosure

None of the authors has any conflict of interest to disclose. 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.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Induction score reflecting the severity of the SE.

Table S2. Categorising behavioural seizures according to modified Racine's scale.

Supplementary methods: Immunohistochemistry

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Figure 1: IL13 producing mesenchymal stem cells or vehicle were injected one week before status epilepticus (SE). Mice were sacrificed for histology at 2-week or 9-week after SE induction. In addition, vEEG recording was performed during chronic epilepsy period (between 6-9 weeks post SE). The sites of cells injections (asterisk) and kainic acid injection (dot) are shown for (B) 9 week and (C) 2 week time point.

Figure 2: Evaluation of SE and seizure burden during chronic epilepsy. (A-C) Graphs comparing (A) SE severity score, (B) duration of SE and (C) number of convulsive seizures during SE between the veh-SE and IL13 MSC-SE group. (D) During chronic epilepsy, three types of pathological events were observed including (D1) seizures accompanied by generalized convulsive activity, (D2) hippocampal paroxysmal discharges (HPDs) with high frequency and low voltage rhythmic discharges (>8-9 Hz) and (D3) seizure like events (SLEs) characterized by low frequency high voltage spikes (typically <5

Hz). Graphs comparing the number of (D) convulsive seizures per day, (F) HPDs per day and (G) number of SLEs per hour in mice with vehicle or IL13 MSC implants. Mean duration of these epileptic events are shown for (H) convulsive seizures, (I) HPDs and (J) SLEs. Number of animals in each group is displayed on X axis for each graph. Data are represented as median $\pm$ interquartile ranges.

Figure 3: Histological evaluations in the ipsilateral hippocampus during chronic period. (A) BFP⁺ cell (arrowheads) after 10w of implantation displayed (B) Iba1⁺ (green) cells and also expressed (C) Arginase1 (red). A merged image is shown in (D). Representative images showing Iba1⁺ cells (green) Arginase 1 (red) and DAPI (blue) in (E) control, (F) veh-SE and (G) IL13 MSC-SE mice. Iba1⁺Arg1⁺ double positive cells were rarely observed at sites away from the IL13 cell grafts. (H) Quantification of number of Iba1⁺ cells in the hippocampus. (I-L) Autoradiographic images showing TSPO expression in three groups and graphs showing its quantification in the hippocampus. *The dotted lines are mean background TSPO signal from healthy mice brains (n=4).* (M-P) Representative images and graphs quantifying F4/80⁺ (red) activated microglia/macrophages in the hippocampus. (Q-T) Representative images showing GFAP⁺ (green) astrogliosis and graphs showing percentage of GFAP⁺ area in the hippocampus. (U-Y) Images showing NeuN⁺ (red) cells in the hippocampus and graph showing neuronal density in GCL (X) and cell loss scores (Y) in the pyramidal CA1 neurons. Insets in E-G and M-O are magnified images. n=5 in control, n=5-6 in veh-SE and n=5-6 in IL13 MSC-SE group. *p<0.05. Data are represented as median $\pm$ interquartile ranges.

Figure 4: Histological evaluations at 2-week after SE induction. MSC-IL13 grafting in mouse hippocampus (A-D). BFP⁺ cells were abundantly present after 3w of implantation (A) and were infiltrated with (B) Iba1⁺ (green) cells that also expressed (C) Arginase1 (red). (E-I) Microglia/macrophage density in the hippocampus away from the cell grafts. Representative images showing Iba1⁺ cells (green) Arginase1 (red) and DAPI (blue) in the hippocampus in (E) control, (F) veh-SE and (G) IL13 MSC-SE animals. Iba1⁺Arg1⁺ double positive cells were rarely observed at sites away from the MSC-IL13 cell grafts. Quantification of number of Iba1⁺ cells in the (H) dentate gyrus (DG) and (I) CA1 area. (J-L) Representative autoradiographic images and (M) graph comparing TSPO expression in the hippocampus between the treatment groups. *The dotted lines are mean background TSPO signal from healthy mice brains (n=4).* TSPO expression was also observed on M2 polarised microglia/macrophages. (N) A representative autoradiographic image is shown that displays strong expression of TSPO at the location of cell graft. High magnification immunofluorescence images show

expression of (O) Ym1 (green), a marker of M2 polarisation and (P) TSPO (red) on the cell graft. A merged image is shown in (Q). Insets in O-Q and E-G are magnified images. n=4 in control, n=6 in veh-SE and n=6 in IL13 MSC-SE group. *p<0.05. Data are represented as median $\pm$ interquartile ranges.

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Author/s:

Ali, I;Aertgeerts, S;Le Blon, D;Bertoglio, D;Hoornaert, C;Ponsaerts, P;Dedeurwaerdere, S

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