Original Article

# Mechanism of the promotion of GEFS+ by the STAT3-mediated expression of interleukin-6

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**Background:** Genetic epilepsy with febrile seizures plus (GEFS+) is generally considered an ion channelopathy. To date, there have been few studies on inflammation associated with various types of epilepsy, and it remains unclear whether the inflammatory mechanism plays a key role in epilepsy.

**Methods:** In order to explore the role of the regulatory mechanism of immune factor expression in the pathogenesis of GEFS+, the present study detected the expression level of relevant immune factors such as interleukin-6 (IL-6) in peripheral blood of GEFS+ mice.

**Results:** The cluster of differentiation $4^+$/cluster of differentiation $8^+$ ($CD4^+$/$CD8^+$) ratio in the GEFS+ mice was decreased, while the signal transducer and activator of transcription 3 (STAT3) was also activated and the IL-6 was upregulated. Inhibit of STAT3 can lead to the GEFS+ asymptomatically due to the downregulated IL-6, IL-1β, and complement factor H (CFH) levels. Suppression of STAT3 can also inhibited the epileptic seizures, the $CD8^+$ T cells were declined after the IL-6 was neutralized.

**Conclusions:** The purpose of this study was to analyze and compare the effect of STAT3 expression and activation differences on GEFS+ attack, and to clarify the relationship between various cytokines and GEFS+ outbreak. Inhibiting the expression of pro-inflammatory factors can further prevent GEFS+ attack, which supports that IL-6 is one of the important factors that aggravate the clinical symptoms of GEFS+. We expected to provide a theoretical basis for immunosuppressive therapy of GEFS+ and a new way for its clinical treatment.

**Keywords:** Genetic epilepsy with febrile seizures plus (GEFS+); interleukin-6 (IL-6); inflammatory state; STAT3; seizures

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## Introduction

Genetic epilepsy with febrile seizures plus (GEFS+) was developed from the concept of generalized epilepsy with febrile seizures (FS) plus, and this condition is usually considered as a genetic "ion channelopathy" (1,2). Sheffer for the first time proposed that GEFS+ is a familial autosomal dominant genetic disease with high incidence in children (3). In 2001, the International League against Epilepsy officially labeled GEFS+ a new type of epilepsy syndrome (4). The genotypes and phenotypes of GEFS+

have certain heterogeneity, owing to the influence of various complex factors, at least 4 genetic loci include SCNB1 (C121W), GABRG2 (K289M and R43Q), SCN1A (R1648H and T875M) and MASS1 (S2652X) (5,6) were related to the onset of GEFS+, it is possible for the heterozygote patients of autosomal dominant inheritance to appear in different phenotypes, involving various epilepsy syndromes, and partial seizures are independent of FS (7). Characteristically, GEFS+ involves an extensive range of clinical manifestations, including the types of epilepsy associated with FS, such as autosomal dominant genetic FS and partial epilepsy with febrile seizures plus (PEFS+) (8,9). Among them, FS is the most common convulsive disease in children, and it usually occurs at the age of 3 months to 5 years old. A sudden outbreak of convulsive symptoms may occur when the body temperature abnormally rises above 38 ℃. The overall prevalence of FS in children is 2–5%, and patients are often found to have a family history of FS, showing a clear hereditary property. Corresponding family-based linkage analysis has shown that the autosomal dominant inheritance of the disease is accompanied by different penetrance, with related gene loci located on chromosomes 19p and 8q13-21 (10). Meanwhile, FS plus (FS+) is featured by afebrile generalized tonic-clonic seizures (GTCS) in children over 6 years old and occasional FS or FS+ with other systemic seizures, such as loss of consciousness, muscle spasm, and atonic seizures. Although less frequent, cases such as afebrile tonic-clonic seizures (TCS), juvenile myoclonic epilepsy, myoclonic absence epilepsy (MAE), and Dravet syndrome may occur in the clinical setting (11,12). Clinical studies have shown that the brain gradually matures as children age, and various clinical symptoms of GEFS+ can disappear spontaneously. However, a small number of patients experience the continuous presence of symptoms into adulthood. Nearly one-third of patients using anti-epileptic drugs (AEDs) experience no improvement in the symptoms of epilepsy, thus aggravating their poor prognosis (13,14). Considering the complexity of clinical manifestations of FS-related epilepsy, especially some severe phenotypes with high difficulty in treatment and poor prognosis, an effective therapeutic approach that can widely deal with and alleviate various clinical symptoms of GEFS+ should be developed to reduce the pain of children and their families.

The pathological process of epilepsy may exhibit an intimate association with a series of cytokines, including inflammatory factors (15). In the pathogenesis of epilepsy with FS, a series of complex inflammatory cascade reactions can result in the accumulation of pro-inflammatory cytokines, blood-brain barrier disruption, and the generalization of neural excitations in the brain, thus increasing the susceptibility of epilepsy and aggravating the damage of nerve cells (16). Moreover, children with recurrent FS may have cellular and humoral immune dysfunction that can enhance the symptoms of epilepsy with FS (17,18). Therefore, immunomodulation and cytokine expression regulation are important to GEFS+ treatment, prognosis improvement, prevention, and treatment. In a study of patients with Rasmussen encephalitis, the activation of microglia, astrocytes, endothelial cells of the blood-brain barrier, peripheral immune cells, and a series of inflammatory mediators could induce chronic encephalitis, and some patients with encephalitis with immune activation were accompanied with epilepsy, similar to autoimmune disease that might result in a high incidence of epilepsy (19). Moreover, limbic encephalitis is an important factor that affects epileptic seizures (20). Therefore, some cytokines and inflammatory signals may be involved in the regulation of some epileptic disorders. Additionally, the classic ketogenic diet therapy for children and adolescents with intractable epilepsy can achieve antiepileptic effect by regulating the expression levels of cytokines (21). In terms of its mechanism of action, the proposed therapy can induce the activation of nuclear hormone receptor peroxisome proliferator-activated receptor α through the high proportion of fatty acid intake. As a transcription regulator, this receptor can downregulate the expression of nuclear factor (NF)-κB-mediated pro-inflammatory factors to inhibit various seizures and thus mediate the treatment of epilepsy by inhibiting the inflammatory response.

In the present study, a mouse model of specific signal transducer and activator of transcription 3 (STAT3) expression inhibition was used to simulate epilepsy with FS+. This study aimed to verify the potential clinical application value of inflammatory cytokine inhibition therapy in GEFS+ treatment by immune blocking agents and targeted therapy for some inflammatory cytokines. By using the multi-omics and chromosomal microarray methods, novel pathogenic factors in the GEFS+ patients may be found in the follow-up studies, and our study may provide a new way for the effective clinical treatment and prevention of GEFS+. We present the following article in accordance with the ARRIVE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-333/rc).

## Methods

### *Construction of the mouse model*

Specific pathogen-free (SPF) one-month-old male C57BL/6 mice (n=16, 8 male and 8 female. The mice were randomly selected for the subsequent experiments) were obtained from Kunshan Yunxuan Laboratory Animal Technology Company Limited (Suzhou, China) and kept under SPF conditions. All animal studies were performed according to the guidelines of the Guidance for Animal Experimentation of Zhejiang Sci-Tech University, and the protocol was approved by the Institutional Animal Care and Use Committee of the Zhejiang Sci-Tech University (No. 201804017). Professor Chen Yuan was aware of the group allocation at the different stages of the experiment. All surgeries were performed under isoflurane anesthesia, and the anesthetized mice were mounted in a stereotaxic frame. The mice received intraperitoneal injection of 55 mg/kg of pentylenetetrazol (PTZ) (22) and were subsequently video-monitored for 20 minutes. The latency to GTCS and deaths were recorded. The epileptiform activity was induced by tail vein injection of glutamatergic analog kainic acid (KA; 10 μM; Sigma-Aldrich, St. Louis, MO, USA). To record the hippocampal electroencephalogram (EEG), we implanted the mice with electrodes and catheters. After the skull was exposed, three holes were drilled on the skull (1.7 mm posterior and 1.0 mm left and right of the bregma; center of the occipital), and the surface of the surgical site was covered and sealed with dental cement. The severity of behavioral seizures was scored according to the modified Racine scale (23) as follows: (I) oral and facial movements; (II) head nodding; (III) forelimb clonus and a lordotic posture; (IV) rearing with forelimb clonus; and (V) rearing, forelimb clonus, and falling. The mice used in our study were euthanized painlessly by decapitation after the experiments. The protocols used in the study were prepared before the study without registration.

### *Inhibitory effect of Stattic on STAT3 phosphorylation*

Stattic is a small non-peptide molecule, which can effectively inhibit the activation and nuclear translocation of STAT3 (24). Stattic can inhibit the binding of DNA to STAT3 dimer selectively and thus suppress STAT3 Tyr705 phosphorylation. In our experiment, Stattic (Selleck, Houston, TX, USA) was selected as the inhibitor of STAT3 activation in mouse samples from the experimental group. It was dissolved into 40 μM mother liquor using dimethyl sulfoxide (DMSO) and refrigerated at –30 ℃ for further use. It was administered through intraperitoneal injection at the concentration of 100 μM/kg. The inhibition effect of STAT3 phosphorylation was verified by western blot analysis.

### *Real-time quantitative polymerase chain reaction*

The total RNA was extracted from the serum samples derived from each group, then the RNA was reverse transcribed to complementary DNA (cDNA) as the templates. Reverse transcription, quantitative polymerase chain reaction (qPCR) normalization, and efficiency correction on β-actin RNA were performed as described in our earlier publication (25). Primers for interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, IL-1β, complement factor H (CFH), and β-actin were obtained from Sangon Biotechnology (Shanghai, China). The primer sequences were as follows: IL-6 F: 5'-TAGTCCTTCCTACCCCAATTTCC-3', R: 5'-TTGGTCCTTAGCCACTCCTTC-3'; TNF-α F: 5'-CCCTCACACTCAGATCATCTTCT-3', R: 5'-GCTACGACGTGGGCTACAG-3'; IL-1β F: 5'-GCAACTGTTCCTGAACTCAACT-3', R: 5'-ATCTTTTGGGGTCCGTCAACT-3'; CFH F: 5'-AGGCTCGTGGTCAGAACAAC-3', R: 5'-GTTAGACGCCACCCATTTTCC-3'; β-actin F: 5'-GGCTGTATTCCCCTCCATCG-3', R: 5'-CCAGTTGGTAACAATGCCATGT-3'.

### *Western blot*

The IL-6, TNF-α, IL-1β, STAT3, and the reference protein [glyceraldehyde-phosphate dehydrogenase (GAPDH)] samples were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto 0.45-μm polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA, USA) for 90 minutes at 350 mA, and then blocked with tris-buffered saline with Tween 20 (TBST) buffer (50 mM Tris-HCl, 150 mM NaCl, and 0.1% Tween-20, pH 7.4) containing 5% non-fat dry milk (w/v) at 4 ℃ overnight. After washing thrice with TBST for 30 minutes, the blots were incubated with rabbit anti-phospho-STAT3 Ab (1:1,000), rabbit anti-TNF-α Ab (1:1,000), rabbit anti-IL-1β Ab (1:1,000), rabbit anti-STAT3 Ab (1:1,000), rabbit anti-IL-6 Ab (1:1,000), or rabbit anti-GAPDH Ab (1:3,000) (Abcam, Cambridge, MA, USA). The blots were washed

thrice with TBST for 30 minutes, and then incubated for 1 hour with the HRP-conjugated goat anti-rabbit IgG Ab (Absin) at room temperature. After washing with TBST, the immunoreactive proteins were visualized using a chemical luminescent immunodetection system (Tanon 4500, Tanon Technology Company, Shanghai, China).

### *Enzyme-linked immunosorbent assay for IL-6 and CFH quantity analysis*

The amount of IL-6 protein in the serum samples and CFH in the hippocampus samples was examined by enzyme-linked immunosorbent assay (ELISA). Each sample was coated onto microtiter wells overnight at 4 ℃. After blocking with 2% bovine serum albumin (BSA) for 2 hours, the wells were incubated with rabbit anti-mIL-6 Ab (or rabbit anti-CFH Ab) for 2 hours at 37 ℃, followed by incubation with the HRP-conjugated goat anti-rabbit Ab (Abcam, China) for 1 hour at 37 ℃. Then, the visualization was developed using a tetramethylbenzidine (TMB) substrate, and the absorbance (A450) value was read by using the Synergy H1 hybrid reader (BioTek Instruments, Winooski, VT, USA).

### *Flow cytometric analysis*

The peripheral blood samples (300 μL each) were derived from the GEFS+ and the control groups using the lymphocyte separation medium to obtain the peripheral blood lymphocytes, and the lymphocytes were analyzed by flow cytometry. The cells were stained for 30 minutes on ice with the following fluorescence-labeled antibodies (1 μg/2×$10^6$ cells) obtained from Abcam: anti-mouse CD3-FITC, anti-mouse CD4-FITC, and anti-mouse CD8-PE. The other two groups (GEFS+ and the control groups) of samples with the same amount of cells were stained for 30 minutes on ice with non-related IgG-FITC as the negative control (background values). The lymphocyte typing was conducted with reference to the product manuals (BBI Life Sciences, Shanghai, China). The cells were washed twice with cold D-Hanks and then more than 10,000 cells from each group were analyzed on BD Accuri C6 [Becton, Dickinson, and Co. (BD), Franklin Lakes, NJ, USA]. Data analysis was performed using BD Accuri C6 software (BD).

### *Statistical analysis*

All experiments included western blots, ELISA and flow cytometry, etc. were repeated at least three times, respectively. The differences between the means of the groups were statistically evaluated using Student's *t*-test, and the data were expressed as the mean ± SD. The criteria for statistical significance used were *P<0.05, **P<0.01, and ***P<0.001 (GraphPad Prism 6.0; GraphPad Software, San Diego, CA, USA). The data were analyzed with the software SPSS 19.0 (IBM Corp., Armonk, NY, USA).

## Results

### *Increased expression of inflammatory-related factors in GEFS+ modeling mice*

After i.p. injection of PTZ, the behavioral seizures of the mouse models were continuously monitored for 6 hours with video recording. The continuous seizure activity of the modeling mice was recorded and it characterized as that persisting beyond 1.5 hours after waking from anesthesia. The secondary generalized convulsive seizures were spasmodically repeated and recorded by the EEG. The control mice (Normal group) received an i.p. injection of isopyknic sterile phosphate buffer saline (PBS). The typical frequent abnormal waves of model mice compared with the control group are shown in *Figure 1A*. The serum samples were derived from the normal mice (n=8) and the mice carrying GEFS+ (n=8). The qPCR and western bolt analysis results showed that the transcriptional levels of IL-6, IL-1β, and TNF-α in GEFS+ mice were significantly increased compared with those in normal mice (*Figure 1B*), the expression level of TNF-α showed no significant change, and the levels of IL-6 and IL-1β in the GEFS+ serum remarkably increased compared with the control sample (*Figure 1C*). According to the results of flow cytometry analysis of peripheral blood lymphocytes in each group, compared to the normal mice [the amount of cluster of differentiation $4^+$ ($CD4^+$) cells was 41.0%±2.7%, n=3], the amount of GEFS+ $CD4^+$ cells (36.0%±3.3%, n=3) and the $CD4^+$/cluster of differentiation $8^+$ ($CD4^+$/$CD8^+$) ratio were decreased, whereas the $CD8^+$ increased in the GEFS+ group (from 18.5%±2.2% to 33.9%±4.7%), with significant statistical difference compared with the control group (normal mice, P<0.01, *Figure 1D*). Hence, with epileptic seizures, a typical inflammatory reaction may occur in mice, and this condition may induce feedback regulation of inflammatory reaction and hence aggravate epileptic seizures.

Moreover, in similarity with the temporal lobe epilepsy

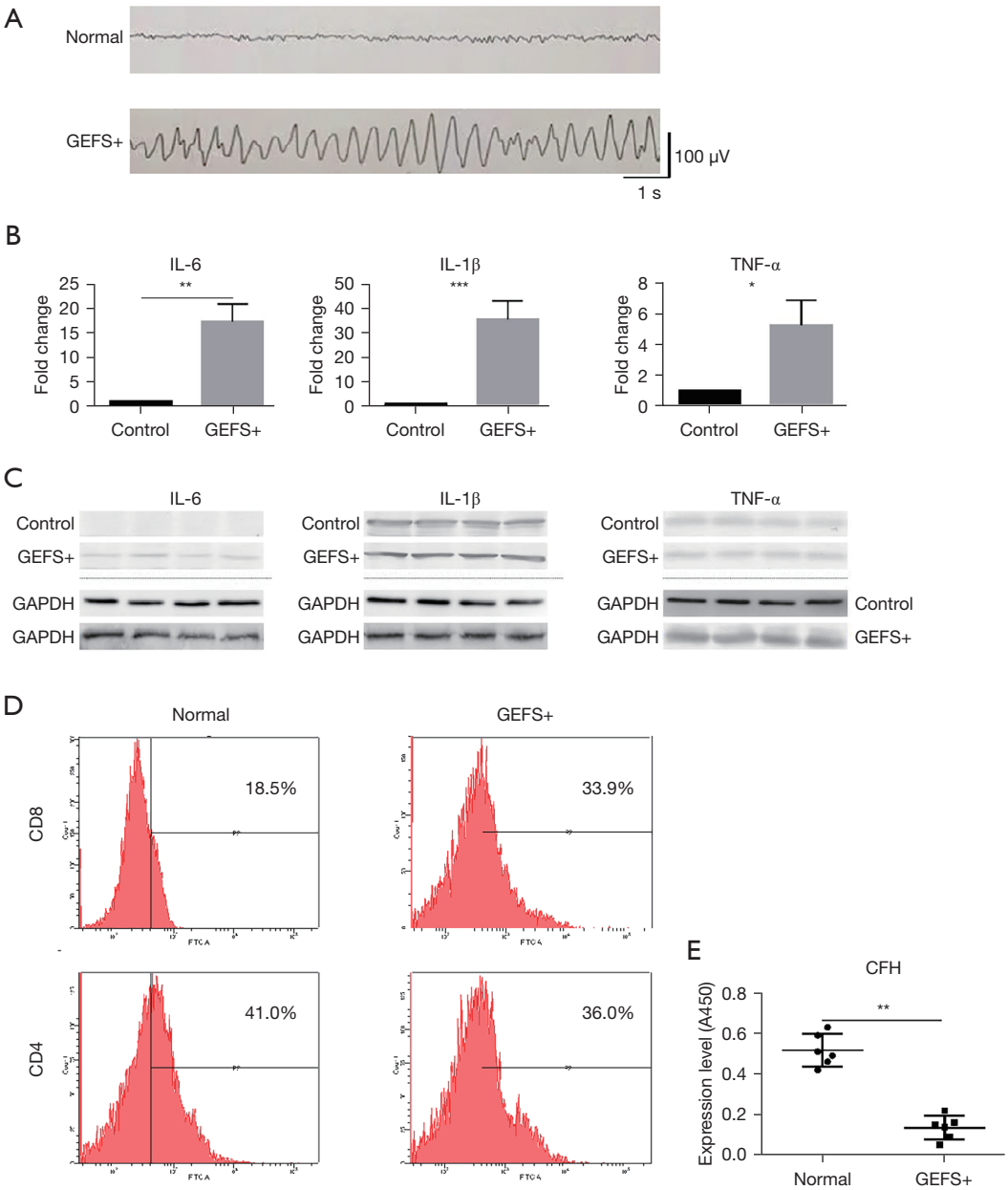


**Figure 1** Pro-inflammatory states occurred after epileptic seizures in GEFS+ mice. (A) EEG recordings from normal and GEFS+ samples after PTZ administration, and epileptiform patterns induced in the limbic region by PTZ; The expression level changes of IL-6, IL-1β, and TNF-α between the control and GEFS+ mice were detected by qPCR (B) and western blot (C) (n=8); The T lymphocyte subsets in the peripheral blood of normal and GEFS+ mice were counted by flow cytometry (the percentages of the T lymphocyte subsets were deducted the background value) (D); (E) CFH protein expression in the hippocampus of the normal and GEFS+ mice were detected by ELISA (n=6). Statistical analysis was conducted by one-way ANOVA. *, P<0.05; **, P<0.01; ***, P<0.001. Values represent means ± SD. The specificity of Abs are presented in Figure S1. GEFS+, genetic epilepsy with febrile seizures plus; IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CFH, complement factor H; EEG, electroencephalogram; PTZ, pentylenetetrazol; qPCR, quantitative polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; ANOVA, analysis of variance; Abs, antibodies; SD, standard deviation.

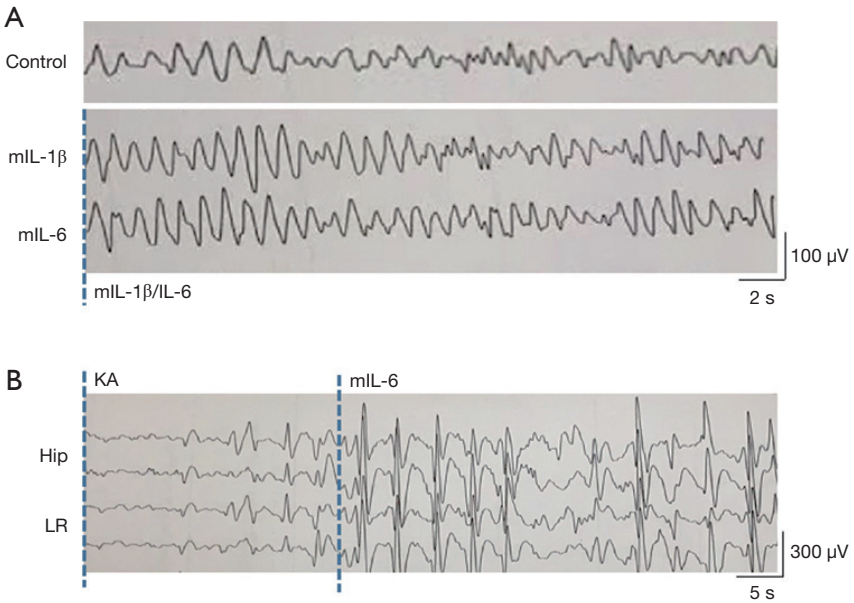


**Figure 2** Epileptiform activity induction in GEFS+ mice by intranasal administration of IL-6 and IL-1β. Sporadic sharp or spike waves can be seen in the control phase, the sharp waves can be seen frequently after administrating IL-6 or IL-1β, and successive abnormal waves appeared with wet-dog shakes (A); epileptiform patterns induced in the Hip (the upper 2 waves) and LR (the under 2 waves) by intranasal administration of IL-6 and i.p. injection of KA (B). mIL-1β, mouse IL-1β; mIL-6, mouse IL-6; KA, kainic acid; Hip, hippocampus; LR, limbic regions; GEFS+, genetic epilepsy with febrile seizures plus; IL-1β, interleukin-1β; IL-6, interleukin-6; i.p., intraperitoneal.

(TLE) rat model, we found that enhancive proinflammatory factors such as IL-1β, TNF-α, and IL-6 could consequently reduce the expression level of CFH in the hippocampus of GEFS+ mice (*Figure 1E*), and thus render IL-6, IL-1β, and CFH the inflammatory loop circuit in epileptic seizure in the GEFS+ model. These results showed that these inflammatory factors have a potential correlation with the symptoms of GEFS+, making them the novel therapeutic targets for GEFS+.

### *Effect of increased IL-6 level on the susceptibility of GEFS+*

The dysfunction of the cell-mediated immunity occurs after repeated FS (15). Therefore, we determined the effect of increased expression levels of IL-6 and IL-1β in the susceptibility of GEFS+ mice to epileptic seizure. To confirm the pivotal role of IL-6 and IL-1β in promoting seizures of GEFS+ mice, 8 GEFS+ mice were intranasally administrated with the recombinant mouse IL-6 (mIL-6) and IL-1β (mIL-1β) (100 ng recombinant cytokines dissolved in 0.9% saline for each mouse), another 8 GEFS+ mice intranasally administrated with 0.9% saline were set as the control group. Then, the behavioral seizures of the mice were continuously monitored for 24 hours with video recording, while the electrode-implanted mice also received EEG monitoring at the same time. Interestingly, although we observed a significant increase in the abnormal wave forms after administrating mIL-6 and mIL-1β (*Figure 2A*), in comparison with the mIL-6-intake mice, convulsive seizures did not increase following mIL-1β administration. Epileptiform activity was induced in the hippocampal and limbic regions by intranasal administration of IL-6 and i.p. injection of KA as detected by EEG monitoring (*Figure 2B*). Thus, IL-6 is a potential target for GEFS+ therapies.

### *Inhibitory effect of STAT3 suppression on IL-6 expression and GEFS+ outbreak*

To explore whether STAT3 could affect seizures by regulating the expression of IL-6 in a GEFS+ mouse model, we administered different concentrations of the STAT3 phosphorylation inhibitor Stattic into GEFS+ mice via i.p. injection. The results of western blot and ELISA showed that the expression of IL-6 significantly decreased under the action of Stattic (*Figure 3A,3B*). With the inhibition of

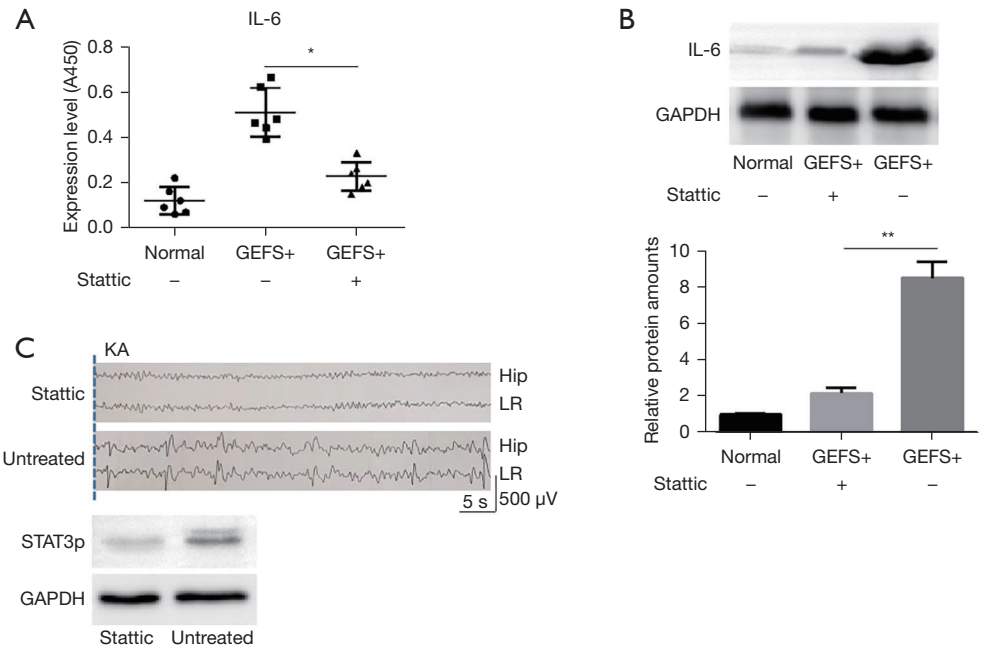


**Figure 3** Suppression of STAT3 inhibited the expression of IL-6 and the epileptic seizures. Detection of the IL-6 protein level in GEFS+ and normal serum upon treated with Stattic (100 μM/kg) by ELISA (A) and western blot (B), and the gray value of the relative signal intensity (IL-6/GAPDH) in western blot analysis was calculated using ImageJ program and depicted in a bar graph. "+" and "–" in the figure above means inhibited by Stattic (+) or not (–). Sporadic sharp waves or spike waves can be found in Stattic-treated GEFS+ mice (C), and the untreated GEFS+ mice showed waves with high amplitude and frequency (Hip, LR). The expression level of STAT3 was deactivated by the administration of Stattic (100 μM/kg) by western blot detection (C). Statistical analysis was conducted by one-way ANOVA. *, P<0.05; **, P<0.01. Values represent means ± SD. The full-length blots are presented in Figure S1. IL-6, interleukin-6; GEFS+, genetic epilepsy with febrile seizures plus; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; KA, kainic acid; Hip, hippocampus; LR, limbic regions; ELISA, enzyme-linked immunosorbent assay; ANOVA, analysis of variance.

IL-6 expression, the abnormal seizure electrical signal of GEFS+ mice was significantly inhibited. Meanwhile, EEG signals showed that compared with STAT3-activated mice, no obvious epileptic activity occurred in the hippocampal and limbic regions of STAT3-inhibited GEFS+ mice, accompanied by delayed and flat successive spike waves (*Figure 3C*).

### *Preliminary study on GEFS+ treatment with targeted inhibition of IL-6 expression*

An anti-IL-6 monoclonal antibody (Abcam) was injected intraperitoneally in GEFS+ model mice to evaluate the effect of the neutralizing activity of proinflammatory cytokine IL-6 on the decrease of the critical value of GEFS+ attack. Following the different injection dosages of IL-6 monoclonal antibody, the abnormal seizure electrical signal of GEFS+ mice was remarkably inhibited. Moreover, EEG signals showed that the successive spike waves of GEFS+ mice with the injection of IL-6 monoclonal antibody became delayed and flat compared with that of mice without IL-6 monoclonal antibody injection (*Figure 4A*), showing the absence of wet-dog shakes in behavior. Correspondingly, after IL-6 was neutralized by anti-IL-6 Ab, the ratio of $CD3^+/CD8^+$ T cells in peripheral blood mononuclear cells (PBMCs) remarkably decreased in the GEFS+ mice according to flow cytometry analysis (*Figure 4B*), and the ratio of CD4/CD8 in anti-IL-6 Ab neutralized GEFS+ mice increased compared with the control group (GEFS+ mice, *Figure 4C*).

The above experiments may lay a foundation for exploring the effective concentration of an IL-6 antibody to inhibit future outbreaks of GEFS+. Its application in the clinical treatment of GEFS+ may provide a new insight into the effective treatment of GEFS+ and inhibition of epilepsy with FS by integrating with joint therapy by using AEDs.

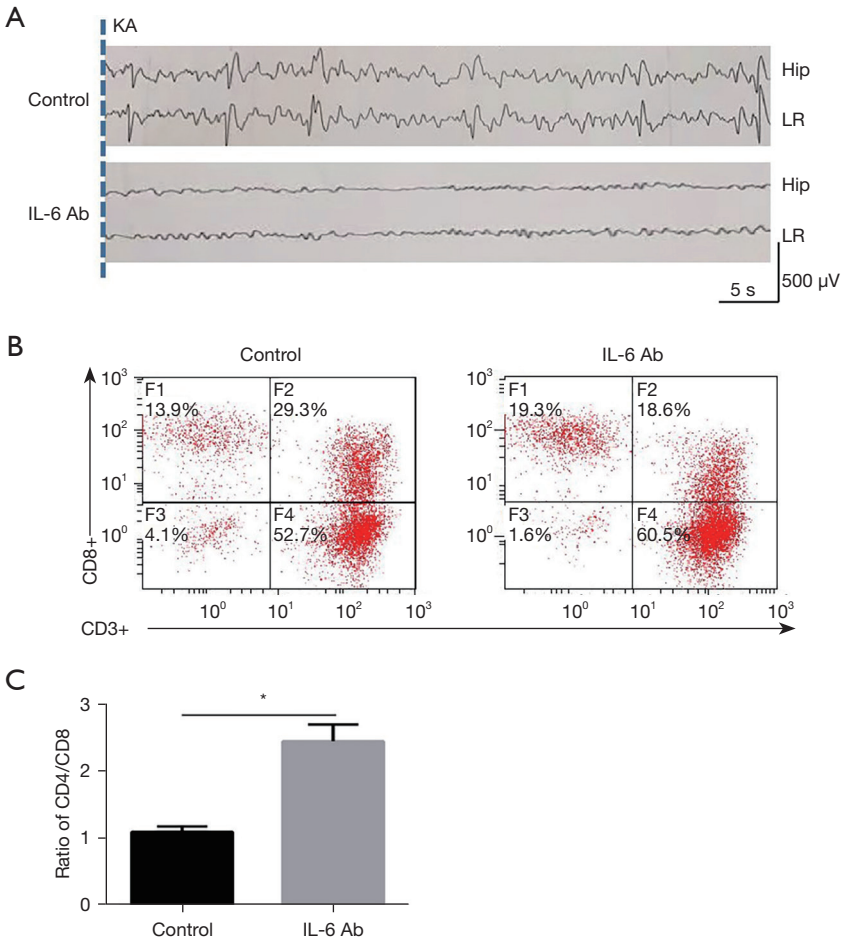


**Figure 4** Neutralization of IL-6 inhibited the epileptic seizures. The anti-IL-6 Ab neutralized GEFS+ mice showed the waves with low amplitude and frequency (A), and sharp waves and spike waves can be found in control mice (Hip, LR); the $CD3^+/CD8^+$ T lymphocytes in PBMCs of control and IL-6 neutralized GEFS+ mice were counted by flow cytometry (B), and the ratios of CD4/CD8 in PBMCs between the control and the IL-6 neutralized GEFS+ mice were also counted (C). Statistical analysis was conducted by one-way ANOVA. *, $P<0.05$. Values represent means ± SD. KA, kainic acid; Hip, hippocampus; LR, limbic regions; IL-6, interleukin-6; Ab, antibody; GEFS+, genetic epilepsy with febrile seizures plus; PBMC, peripheral blood mononuclear cell; ANOVA, analysis of variance; SD, standard deviation.

## Discussion

At present, there is still insufficient research on the inflammatory status in various types of epileptic seizures; it is unclear whether the inflammatory mechanism plays a key role in epilepsy. Meanwhile, there have been few studies on the severity of epileptic symptoms caused by the differences in the expression levels of individual inflammatory factors, especially IL-6 (26), Choi *et al.* (27) suggested that the postictal serum IL-6 levels were not significantly associated with the genotypes of IL-6-572 (rs1800796) in GEFS+, no more potential genetic risk factors in postictal IL-6 were found in the previous studies. Accordingly, this study analyzed the clinical data of early GEFS+ to explore its immunological characteristics and the biological mechanism of promoting GEFS+ attack. In our experiment, epilepsy with FS+ was stimulated by a gene mutation-based mouse model, followed by the application of immune blocking agents and targeted therapy based on some inflammatory cytokines, which was designed to verify the potential clinical application value of inflammatory factor suppressive therapy in GEFS+ treatment.

As evidenced by prior research (28), epileptic seizure can induce the activation of glial cells and other non-neuronal cells, and then stimulate the expression of a large number

of inflammatory-related factors, such as IL, interferon, and TNF. It may further promote the inflammatory immune environment in the course of epileptic seizures. According to the published clinical research (29), glucocorticoid and adrenocorticotropic hormones could play an effective therapeutic role in intractable epilepsy in children, and a great deal of glial cell hyperplasia was found in the epileptogenic focus of the brain. This may suggest that inflammation might play an essential role in inducing the pathogenesis of epilepsy and affecting its severity. Furthermore, Lehtimäki *et al.* (30) discovered in their experiment that the expression of IL-6R and the level of its signal transduction factor Gp130 were significantly increased in the forebrain of epileptic rats. Intranasal administration of IL-6 could also increase the severity of induced seizures in rats (31). Additionally, through the analysis of clinical data, serum IL-6 level was shown to be significantly higher in chronic TLE patients than that of normal group, which also exhibited an obvious increased trend compared with other epilepsy patients without TLE (32), suggesting that the type of epileptic seizure may have an impact on the level of IL-6. Nevertheless, it remains unknown whether serum IL-6 level changes during epileptic seizures in patients with GEFS+ and whether STAT3-IL-6 can promote epileptic seizures in those patients. In our study, a mouse GEFS+ model was established in C57BL/6 mice by using the epileptogenic agent PTZ. The corresponding results revealed that the levels of IL-6 and IL-1β in serum were increased significantly along with typical epileptic versus after KA stimulation (*Figure 1C*). Meanwhile, according to the analysis of the types of peripheral blood lymphocytes in GEFS+ mice, the proportion of $CD4^+$ lymphocytes decreased significantly, while that of CD8+ lymphocytes increased in GEFS+ mice (*Figure 1D*). This may reveal the presence of typical inflammatory reaction in mice with epileptic seizures. Besides, intranasal administration of IL-6 to GEFS+ mice further increased the susceptibility of epilepsy, suggesting that inflammatory response may play a feedback regulation role to aggravate epileptic seizures.

Furthermore, in our experiment, following the injection of Stattic, a specific STAT3 phosphorylation inhibitor, into GEFS+ mice, the abnormal electrical signals of epileptic seizures in GEFS+ mice were also significantly inhibited with the suppression of IL-6 expression (*Figure 3*). At the same time, there was an inhibition of the epileptic activity of mice to a certain extent. A similar phenomenon was observed in GEFS+ mice after intraperitoneal injection of an anti-IL-6 monoclonal antibody. The above results may suggest that the upregulation of IL-6 mediated by STAT3 may be involved in the mechanism of epileptic seizures in GEFS+ mice. Significantly, our study preliminarily reveals the mechanism of some certain cytokines in promoting GEFS+ outbreak, and elucidates the molecular mechanism of inflammatory signaling pathway STAT3-IL-6 involved in the regulation of GEFS+ pathogenesis. This study revealed that the monoclonal antibody can inhibit the expression of pro-inflammatory factors and further prevent GEFS+ attack, which supports that IL-6 is one of the important factors that aggravate the clinical symptoms of GEFS+. The findings of the present study may provide some valuable experimental evidence for further understanding of the key role of cytokines in the development of GEFS+.

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## Footnote

*Reporting Checklist:* The authors have completed the ARRIVE reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-333/rc

*Data Sharing Statement:* Available at https://tp.amegroups.com/article/view/10.21037/tp-22-333/dss

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-22-333/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Animal Care and Use Committee of the Zhejiang Sci-Tech University (No. 201804017). All animal studies were performed according to the guidelines of the Guidance for Animal Experimentation of Zhejiang Sci-Tech University.



 

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