Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

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

Original Article

Diminished toll-like receptor response in febrile infection-related epilepsy syndrome (FIRES)

Meng-Ying Hsieh [a,b,d,g,*], *Jainn-Jim Lin* [c,g], *Shao-Hsuan Hsia* [c,g], *Jing-Long Huang* [d,e,g], *Kuo-Wei Yeh* [e,g], *Kuei-Wen Chang* [a,f,g], *Wen-I. Lee* [d,e,g,*]

[a] *Division of Pediatrics, Chang Gung Memorial Hospital at Taipei, Taipei, Taiwan*
[b] *Division of Pediatric Neurology, Department of Pediatrics, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[c] *Division of Pediatric Critical Care, Department of Pediatrics, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[d] *Primary Immunodeficiency Care and Research (PICAR) Institute, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[e] *Department of Pediatrics, New Taipei Municipal TuCheng Hospital, New Taipei City, Taiwan*
[f] *Division of Pediatric Gastroenterology, Department of Pediatrics, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan*
[g] *College of Medicine, Chang Gung University, Taoyuan, Taiwan*

ARTICLE INFO

Article history:
Received 2 December 2019
Accepted 3 May 2020
Available online 29 May 2020

Keywords:
Refractory seizure
Febrile infection-related epilepsy syndrome (FIRES)
Toll-like receptor
Monocyte-derived dendritic cells (MDDCs)
Status epilepticus

ABSTRACT

Background: Defective human TLR3 signaling causes recurrent and refractory herpes simplex encephalitis/encephalopathy. Children with febrile infection-related epilepsy syndrome with refractory seizures may have defective TLR responses.

Methods: Children with febrile infection-related epilepsy syndrome were enrolled in this study to evaluate TLR1-9 responses (IL-6, IL-8, IL-12p40, INF-α, INF-γ, and TNF-α) in their peripheral blood mononuclear cells (PBMCs) and monocyte-derived dendritic cells (MDDCs), compared to those with febrile seizures and non-refractory epilepsy with/without underlying encephalitis/encephalopathy.

Results: Adenovirus and enterovirus were found in throat cultures of enrolled patients (2–13 years) as well as serologic IgM elevation of mycoplasma pneumonia and herpes simplex virus, although neither detectable pathogens nor anti-neural autoantibodies in the CSF could be noted. Their PBMCs and MDDCs trended to have impaired TLR responses and significantly lower in cytokine profiles of TLR3, TLR4, TLR7/8, and TLR9 responses but not other TLRs despite normal TLR expressions and normal candidate genes for defective TLR3

* Corresponding author. Primary Immunodeficiency Care and Research (PICAR) Institute, Chang Gung Memorial Hospital at Linkou, 5, Fusing St., Gueishan, Taoyuan 333, Taiwan.
E-mail addresses: survanta@gmail.com (M.-Y. Hsieh), wen2707@gmail.com (W.-I. Lee).
Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2020.05.007

signaling. They also had decreased naïve T and T regulatory cells, and weakened phagocytosis.
Conclusion: Children with febrile infection-related epilepsy syndrome (FIRES) could have impaired TLR3, TLR4, TLR7/8, and TLR9 responses possibly relating to their weakened phagocytosis and decreased T regulatory cells.

At a glance of commentary

Scientific background on the subject

The mechanisms of refractory seizures and the causes in children with febrile infection-related epilepsy syndrome (FIRES) remain unclear and immune dysfunction may play a role.

What this study adds to the field

FIRES children have impaired TLR 3, 4, 7/8 and 9 responses that may relate to weakened phagocytosis and decreased T regulatory cells. Such TLR impairments can persist for more than 12 months.

Febrile infection-related epilepsy syndrome (FIRES) is a catastrophic epileptic encephalopathy with known febrile infection preceding the onset of refractory status epilepticus, but without evidence of identified infectious encephalitis. It is frequently associated with high mortality and morbidity, especially in areas where enterovirus, herpes simplex virus and influenza infections are endemic [1–3]. The pathogenic mechanisms of FIRES including infection- or inflammatory-mediated process, metabolic disorders, monogenic epilepsy genes, genetic predisposition, and autoimmunity have been proposed but the definite cause remains unknown. There are increasing evidences suggest that the mechanism is caused by autoimmunity due to autoantibodies production against the neuronal surface of N-methyl-D-aspartate receptors (NMDARs), glutamic acid decarboxylase (GAD), or intracellular molecules such as amphiphysin, CRMP5 (Anti-CV2), and ANNA-1 (Anti-Hu). Some cases with underlying malignancy present as paraneoplastic syndromes and induce autoantibodies production against neurons leading to refractory seizures [4]. However, the mechanism of refractory seizures remains unclear in over half of all cases [2,4].

Experimental mice lacking IFN cytokines of IFN-α, IFN-β, and IFN-λ are predisposed to several viral infections [5]. In human and mice anti-viral defense systems [6–8], Toll-like receptor (TLR)3, TLR7, TLR8, and TLR9 can induce IFN cytokine production via the TLR-IFN pathway to resist various viral infections [9]. Within the human TLR-IFN downstream pathway, autosomal recessive UNC93B1 [10] and autosomal dominant TLR3 deficiencies [11] were firstly found to predispose healthy patients to herpes simplex encephalitis (HSE), suggesting a redundancy in immunity to most other viral infections [11–15]. After that, defective downstream TLR3 signaling caused by TRIF, TRAF3 and TBK1 mutations was subsequently discovered [11–19].

The aim of this study was to determine whether children with FIRES have defective TLR pathways unable to eradicate infection-related triggering factors and inhibit epileptogenesis, therefore leads to refractory seizures. We investigated their TLR responses in primary peripheral blood mononuclear cells (PBMCs) and secondary monocyte-derived dendritic cells (MDDCs) [20,21] to convince the consistent findings.

Methods

Patients

We enrolled FIRES patients included the criteria of (1) fever (≥38 °C) during the acute phase of illness, (2) symptoms of consciousness changes in the acute phase of illness, (3) acute onset of seizures at least 2 days after an antecedent febrile infection in previously healthy children, (4) seizures were so frequent and evolved into refractory status epilepticus without a latent period, (5) absence of pathogens identified in the CSF, and (6) prolonged duration of acute illness lasting more than two weeks. Magnetic resonance imaging (MRI) study were arranged to evaluate if there were abnormal signal changes. The presence of associated pathogens were investigated in the blood, cerebrospinal fluid (CSF), throat and anal swab cultures, paired serology and pathogenic PCR, including HSV, EBV, CMV, influenza, adenovirus, varicella, and mycoplasma pneumoniae. Anti-neural autoantibodies consisting of metabotropic glutamate receptor 5 (mGluR 5), dipeptidyl-peptidase-like protein-6 (DPPX), and γ-aminobutyric acid-A receptor (GABAAR). N-methyl-D-aspartate receptor (NMDAR), γ-aminobutyric acid-B receptor (GABABR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), leucine-rich glioma inactivated protein 1 (LGI1), and contactin-associated protein-like 2 (CASPR2) were detected.

Those patients with febrile seizures, non-refractory epilepsy with and without underlying encephalitis/encephalopathy were enrolled as the controls. The Institutional Review Board (IRB) of Chang Gung Memorial Hospital approved this study, and all of the participants provided written informed consent.

Basic immunologic function

Peripheral blood samples (15 ml) from the enrolled patients and age-matched controls were drawn and analyzed within 24 h of the onset of refractory seizures and before the initiation of intravenous immunoglobulin (IVIG) and

methylprednisolone pulse therapy. PBMCs were isolated by Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) centrifugation. RPMI 1640 supplemented with 10% fetal calf serum (FCS; Hyclone, UT), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (complete medium) was used as complete culture media for further analysis.

To induce lymphocyte proliferation, PBMCs (10^5/well) were incubated with the indicated concentrations of PHA, ConA, PWM or/and CD3CD28 for 3 days, or Candida antigen and BCG vaccine for 7 days. Lymphocyte subsets (all antibodies purchased from Pharmingen, San Jose, CA) were assessed by flow cytometry as previously described [22–24], including CD3+, CD4+, CD8+, CD19+, CD45, CD45RO+, CD45RA+, CD27+, CD16 + CD56+ (natural killer cells) and T regulatory cells (Treg; CD4+FOXP3+).

To assess the effect of surface TLR responses on phagocytosis, pathogenic particles of BODIPY-labeled *Staphylococcus aureus* (through TLR2) or *Escherichia coli* (TLR4) (Molecular Probes, Eugene, OR) were incubated in a constant ratio of 10 to 1 polymorphonuclear cells (2×10^6) for 2 h at room temperature in the presence of their own sera (for opsonization) as previously described [25].

Expression of TLR1-9 on PBMCs

Antibodies for human TLR and isotype controls were purchased from Imgenex Corp. (phycoerythrin-conjugated [PE]-conjugated TLR1-8) and eBioscience (PE-conjugated TLR9, San Diego, CA). TLR1, TLR2, TLR4, TLR5, and TLR6 were detected using surface staining, and TLR3, TLR7, TLR8 and TLR9 by intracellular staining. For intracellular staining, PBMCs were fixed in 4% formaldehyde for 15 min and permeabilized with 0.1% saponin for 30 min on ice. For both protocols, the cells were incubated with the corresponding TLR antibodies. A total of 10,000 viable lymphocytes were collected and analyzed by flow cytometry (FACSCalibur, BD Biosciences Corp., San Jose, CA).

TLR ligand stimulation

To determine TLR responses, two stimulating dosages of TLR1-9 agonists or media only at a log range (except for TLR4 agonist) were chosen to test the dose-dependent effect on PBMCs (3×10^5) in 100 μl complete media at 37 °C for 24 h: 1.0, 10 ug/ml Pam3CSK4 (TLR1/2 ligand, Invivogen, San Diego, CA); 1.0, 10 ug/ml poly (I:C) (TLR3 ligand, Sigma-Aldrich Co., St. Louis, MO); 1.0 ug/ml LPS (TLR4 ligand, Invitrogen, San Diego, CA); 0.1, 1.0 ug/ml flagellin (TLR5 ligand, Calbiochem Corp., San Diego, CA); 0.1, 1.0 ug/ml zymosan (TLR2/6 ligand, Invitrogen, San Diego, CA); 1.0, 10 ug/ml R848 (TLR7/8 ligand, Invitrogen, San Diego, CA); and 1.0, 10 ug/ml ODN 2216 (TLR9 ligand, Coley Pharmaceuticals Wellesley, MA). IL-6, IL-8, IL-10, IL-12, TNF-α., IFN-γ (R & D Systems, Minneapolis, MN), INF-α and INF-β (TFB, Fujirebio, Inc., Tokyo) production was assessed by ELISA as previously described [26,27]. Every condition was performed in duplicate. $P < 0.05$ was thought of significance by unpaired t tests (Graph Pad 4.0).

Development and stimulation of monocyte-derived dendritic cells

Compared to PBMCs, MDDCs have the potential to mount the TLR response [28–30]. Thus, CD14+ monocytes were purified using a Monocyte Isolation Kit II (BD Biosciences Corp.), grown in GM-CSF (50 ng/ml) and IL-4 (25 ng/ml) in culture media (each 10^5/ml) for 6 days and replaced every other day. CD14-FITC, HLA-DR-PE, CD11b-PE and CD80-PE (all punched from BD Biosciences Corp.) were utilized to evaluate the status of maturation in monocyte-derived dendritic cells (MDDCs) [28,31] which resulted in increased expressions of HLA-DR, CD80, and CD11b but a decreased expression of CD14 [Fig. 1]. The expression of TLR and cytokine production from the MDDCs stimulated by TLR3, TLR4, TLR7/8, and TLR9 agonists were also evaluated to investigate whether these results paralleled with those in the PBMCs.

Results

Patient characteristics

Five (one female) FIRES children were enrolled. All of them had episodes of status epilepticus, abnormal imaging results (Fig. 2), and/or epileptiform discharges in electroencephalography (EEG) (Table 1). None of the patients had neutropenia, lymphopenia, or hypogammaglobulinemia, however two had naïve CD4+ lymphopenia and four had lower Treg cells.

All the patients had elevated D-dimers and liver enzymes due to hypoxia during the frequent seizure episodes. CSF cultures and PCR amplifications did not reveal any pathogens despite of mild CSF pleocytosis (WBC >10/ml) was noted in three patients. However, adenovirus, enterovirus (in two throat cultures), mycoplasma pneumoniae, and HSV (in two serologic IgM elevations) were detected in three patients and sub-clinical infection was not excluded in the other two patients. Aside from AEDs, IVIG and methylprednisolone pulse therapy followed by prednisolone 0.5–2 mg/kg/day for 1–4 months decreased the frequency of seizures and interrupted the status epilepticus, especially in patient 3 who had uncal and tonsillar herniation.

TLR expressions and responses in PBMCs

The expressions of TLR 1–9 in the patients were similar to those of the controls, including those with febrile seizures with or without underlying encephalitis/encephalopathy (as a representative demonstration in Supplemental Fig. 1). INF-β was not detectable in any patient. The TLRs responses generally trended to decrease and be significantly lower in TLR3 (in terms of IL-6, IL-8, INF-α, and INF-γ), TLR4 (INF-γ), TLR7/8 (INF-α, INF-γ and IL-12p40) and TLR9 (IL-6, INF-α, and INF-γ) pathways by their respective agonists while compared to those of the controls (Fig. 3, Supplemental Table 1).

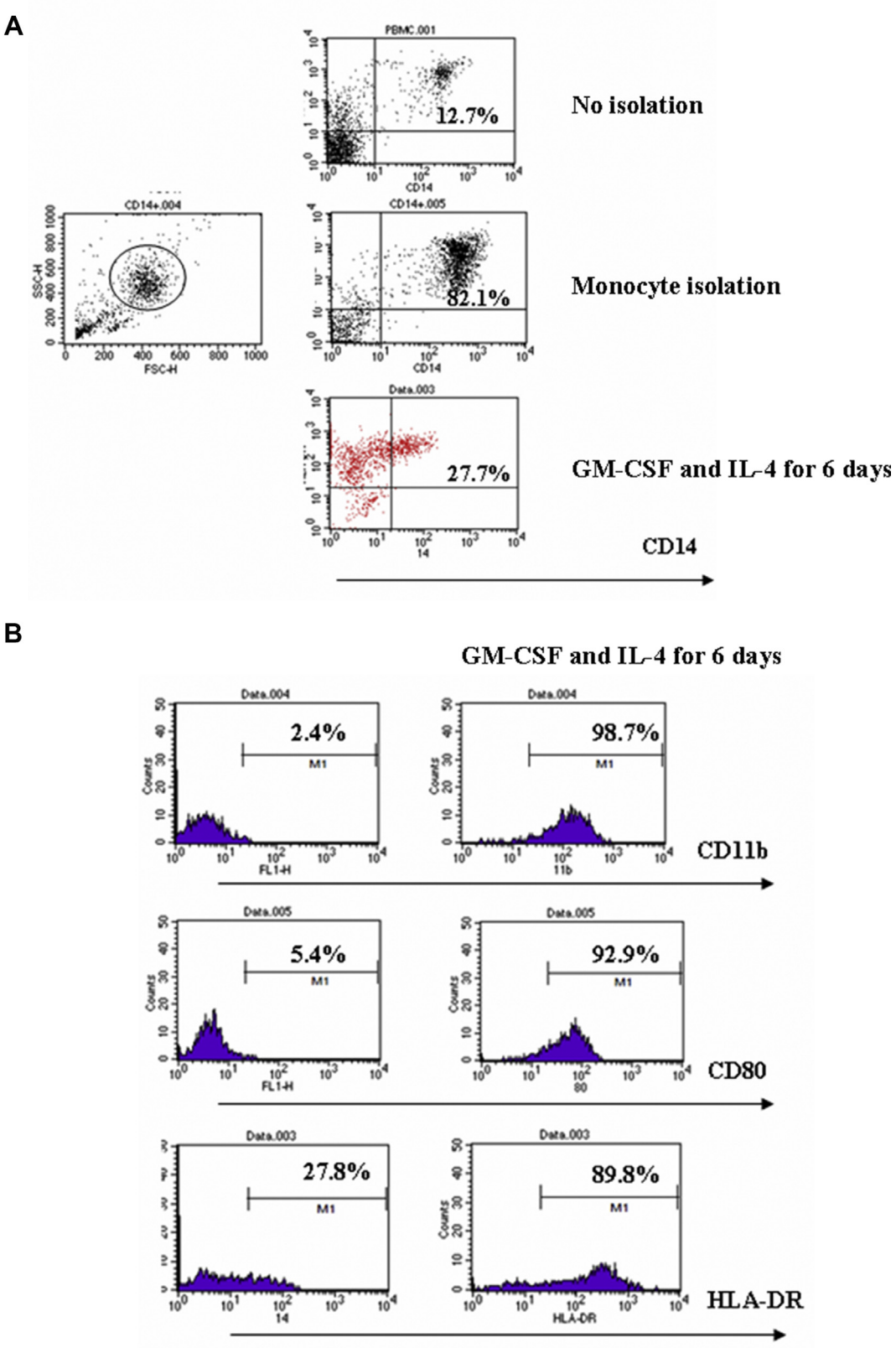

Fig. 1 Using monocyte (CD14) isolation, more homogenous monocytes (82.1%) were obtained from PBMCs (12.7%). **(A)** The percentage of CD14+ staining in isolated monocytes decreased in the monocyte-derived dendritic cells (MDCCs). **(B)** In contrast to CD14 expression, the expressions of CD11b, CD80 and HLA-DR (MHC class II) obviously increased in parallel with the maturation of MDDCs after co-culture with GM-CSF and IL-4 for 6 days. The results were similar in both patients and controls.

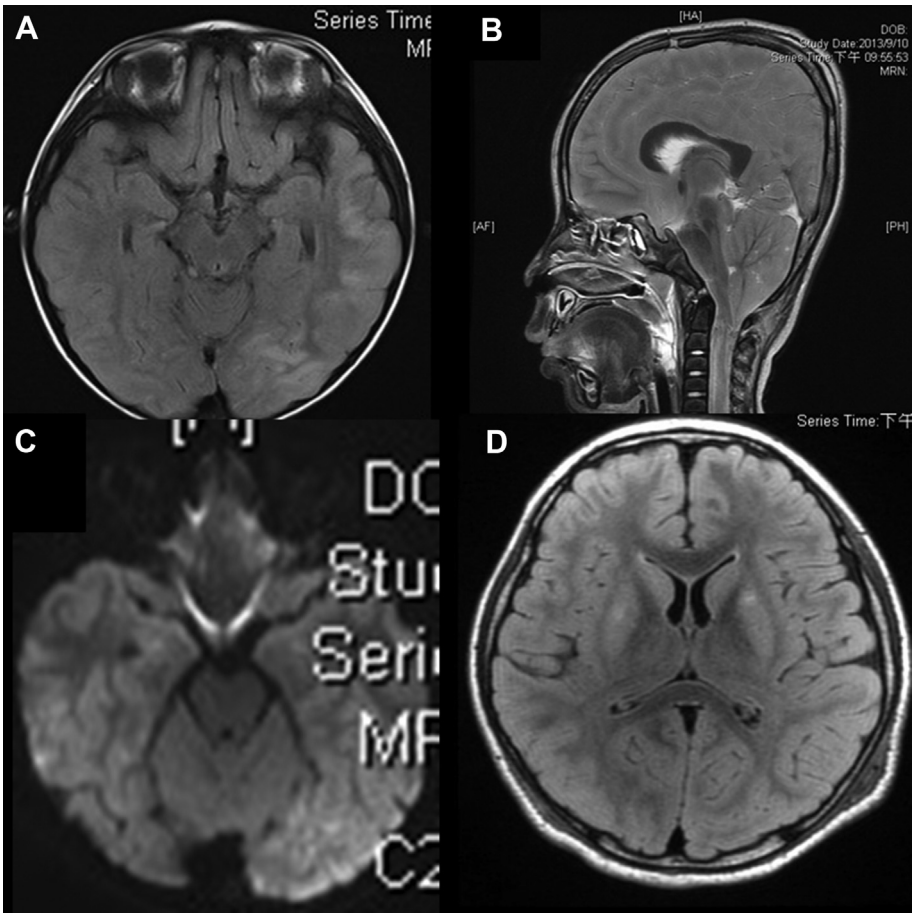

Fig. 2 Representative encephalitis/encephalopathy image in CT showed that (A) multiple asymmetrical areas (T2-hyperintensity and subtle T1-hypointensities) in the temporal and occipital cortical lobes in case 1; (B) Bilateral uncal and tonsillar herniations compressing the brain stem through foramen magnum in case 3; (C) Multiple asymmetrical areas in the bilateral caudate heads, lentiform nuclei, posterior thalamus and multiple cortical areas of bilateral frontal, parietal, occipital and subinsular regions in case 4; (D) Hyperintensities in the bilateral putamen, right hipppocampus, and bilateral anterior temporal cortex with interval atrophic change; Cavity change of the bil putaminal lesions in case 5.

Maturation of MDDCs and cytokine production

Interestingly in blank medium, the production of IL-6 and IFN-γ was significantly lower and the others trended to be relatively low in the MDDCs, implying insufficient MDDC development. TLR3 (in term of IL-6, IL-8, INF-α, INF-γ and TNF-α), TLR4 (IL-12p40), TLR7/8 (INF-α and IL-12p40), and TLR9 (IL-6, IL-8, INF-α, and IL-12p40) responses also significantly decreased (in Fig. 4 and Supplemental Table 2). The intracellular TLR3, TLR7/8, and TLR9 expressions in the PBMCs and MDDCs were similar (71–99%) in the patients and controls (in Fig. 5 and Supplemental Fig. 1).

The TLR expressions and functions of the PBMCs and MDDCs were evaluated again when the patients were in the seizure-free status after 12 months' treatment. Their expressions and cytokine profiles from the TLR responses in stable status were consistent with those in the refractory status (data not shown).

Discussion

Children with FIRES showed impaired TLR3, TLR4, TLR7/8, and TLR9 responses. These observations in our patients inferred that anti-RNA viral cytokine profile of TLR responses to the associated pathogens of adenovirus, enterovirus, and herpes simplex behaving as TLR3, TLR7/8 and TLR9 agonists were down-regulated [29,30,32,33]. Expectedly, decreased pro- and inflammatory cytokines of IL-6, IL-8, INF-α, and TNF-α through these impaired TLR3, TLR7/8, and TLR9 pathways could attenuate phagosome-associated responses [34] reflecting on their diminished phagocytosis (i.e., TLR2 for *S. aureus* and TLR4 for *E. coli* in Table 1). As well as that lower number of naïve T cells increased susceptibility to viral infections, weakened phagocytosis was not able to effectively eradicate pathogens in time and therefore accumulate damaged debris, especially virus pathogens. By the mimicking mechanisms, prolonged exposure to such

Table 1 Clinical and significant laboratory features in FIRES patients with refractory seizures.

Gender	Case 1/M	Case 2/F	Case 3/M	Case 4/M	Case 5/M
Age	2Y11M	2Y9M	3Y3M	13Y2M	7Y1M
Fever	+	+	+	+	+
Status Epilepticus	+	+	+	+	+
CSF					
WBC/RBC (5–10 high power view)	3/0	**12**/1	7/0	**23**/1	**18**/3
Sugar	42	51	57	63	48
Protein (8–32 mg/ml)	19	29	25	**47**	**52**
IgG index (< .7)	0.37	0.42	0.39	0.41	0.37
Identified pathogens	Negative	Negative	Negative	Negative	Negative
Imaging findings [MRI]	Encephalitis[b]	Non-significant	Encephalitis[b] Uncal and tonsillar herniation	Encephalitis[b]	Post-encephalitis atrophy
EEG					
Cortical dysfunction	+		+		+
Focal epileptiform		+		+	+
Hematology[d]					
WBC (1000/ul)	1.5 (5.0–15.5)	17.0 (5.0–15.5)	1.27 (5.0–15.5)	7.9 (4.5–13.5)	11.7 (4.5–13.5)
S/L	43/27	80/10	73/21	78/16	73/22
Hb (g/dL)	10.9 (11.5–12.5)	13.1 (11.5–12.5)	14,4 (11.5–12.5)	13.6 (13.0–14.5)	15.5 (11.5–13.5)
PLT (1000/ul)	338 (150–350)	242 (150–350)	482 (150–350)	146 (150–350)	260 (150–350)
Biochemistry					
CRP	78.2	2.8	14.8	86.5	4.0
D-dimer (< 250 mg/ml)	**934**	**741**	**1172**	**3269**	**2047**
GOT/GPT (60/40 U/L)	35/19	37/12	28/21	**134/77**	**163/125**
BUN/Cr (mg/dL)	3.1/0.35	8.4/0.2	18.4/0.4	23.9/1.3	7.3/0.3
Ca/P/Mg (mg/dL)	9.1/4.7/1.6	9.7/3.2/1.7	9.5/3.8/1.9	8.2/4.0/1.6	9.3/3.8/1.8
Immunologic data[c]					
IgG	650	924	1090	959	1042
IgG2	160	253	153	259	208
IgA	61.8	85.9	147	162	114
IgM	126	141	152	52.7	102.3
IgE	1170	UN	897	80.7	42.7
CD4CD45RA% (> .15%)	26.5	16.2	**8.4**	**10.8**	24.2
CD4CD45RO%	8.7	10.1	8.3	10.0	10.5
CD4FOXP3% (2–8%)	**1.2**	**1.4**	**0.8**	**0.7**	2.1
CD19CD27%	4.8	2.9	5.4	1.4	2.3
Memory T%	8.5	10.2	8.5	10.2	9.8
CD19%	32.1	37.2	62.7	39.8	18.2
Memory B%	4.9	3.0	5.9	1.5	2.4
NK%	3.7	2.8	1.8	4.0	5.1
Phagocytosis					
E coli (87–99%)	84	62	57	79	71
Staphy aureus (92–99%)	75	48	43	64	66
Associated virus					
Throat culture	Adenovirus	Enterovirus			
Serology IgM		Mycoplasma	Mycoplasma, HSV		

Detectable autoantibodies:					
Anti-TPO	−	−	−	+	−
Anti-GAD	−	−	−	−	−
Anti-neuro[a]	−	−	−	−	−
Admission (days)					
Intense care unit	11	5	75	32	128
General unit	26	24	9	8	41
IICP event	−	−	+	+	+ (hemorrhage)
Treatment					
Anti-epileptic drugs (AEDs)	Phenobarbital, phenytoin, levetiracetam	Phenobarbital, phenytoin, levetiracetam	Phenobarbital, phenytoin, valproate	Phenobarbital, phenytoin, levetiracetam	Valproate, levetiracetam, topiramate, clonazepam, oxcarbazepine,
Steroids	pulse	pulse	pulse	pulse	pulse
IVIG	+	+	+	+	+
Alternative					**Hypothermia**
Seizure frequency (per week)					
The first week	**21**	**24**	**38**	**21**	**29**
The week of the last visit	3	4	5	2	3

The numbers underlined in bold italics indicate that they are higher than the normal range but below CD4CD45RA naïve cells.

[a] Anti-neural autoantibodies included metabotropic glutamate receptor 5 (mGluR 5), dipeptidyl-peptidase-like protein-6 (DPPX), and γ-aminobutyric acid-A receptor (GABAAR). N-methyl-D-aspartate receptor (NMDAR), γ-aminobutyric acid-B receptor (GABABR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), leucine-rich glioma inactivated protein 1 (LGI1), and contactin-associated protein-like 2 (CASPR2).

[b] Cortical fluid-attenuated inversion recovery (FLAIR) or T2 multifocal lesions consist with encephalitis/encephalopathy.

[c] All patients had normal lymphocyte proliferation to mitogens (PHA, ConA, and PWM), antigens and CD3CD28).

[d] The standard range is based on Aquino J: The Harriet Lane Handbook, Philadelphia, Mosby, 2009.

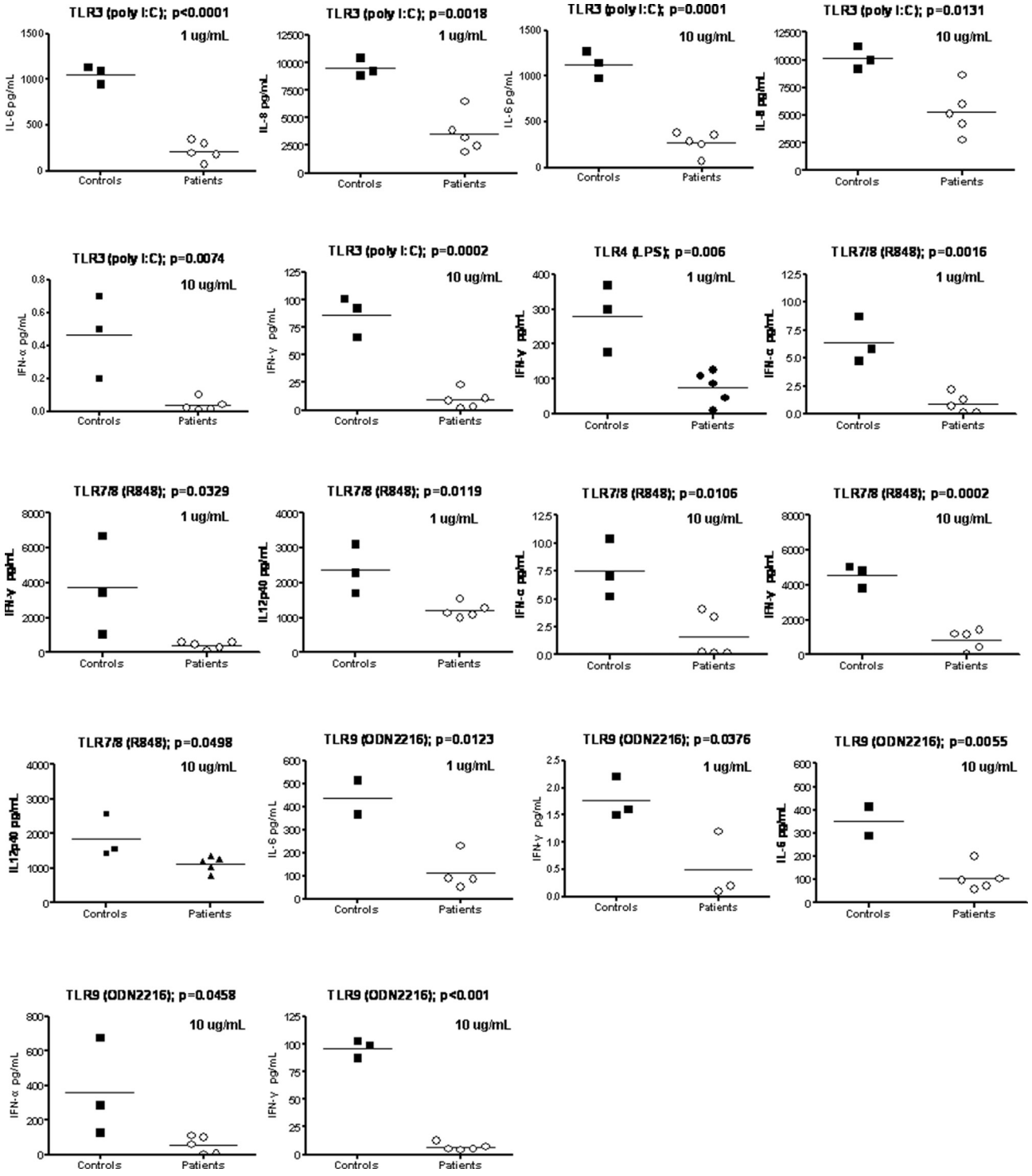

Fig. 3 Significantly lower ($p < 0.05$; unpaired test) scatter diagrams of cytokine production (pg/ml) through TLR3, TLR4, TLR7/8, and TLR9 signaling in PBMCs from children with febrile infection-related syndrome.

damaged debris behaving as epitopes cross-reactive to neural components could induce autoimmunity to central neurons. Furthermore, lower number of Treg cells in our patients lack sufficient suppression to counteract such unwanted autoimmune or/and inflammation responses [35]. These findings could partially explain why adenovirus, enterovirus, herpes simplex virus, and mycoplasma pneumoniae were detectable in the throat and serology rather than in the CSF, and how these pathogen-debris mimicking epitopes attract autoantibodies into central neural system to attack central neurons, and subsequently orchestrate refractory seizures [34–36].

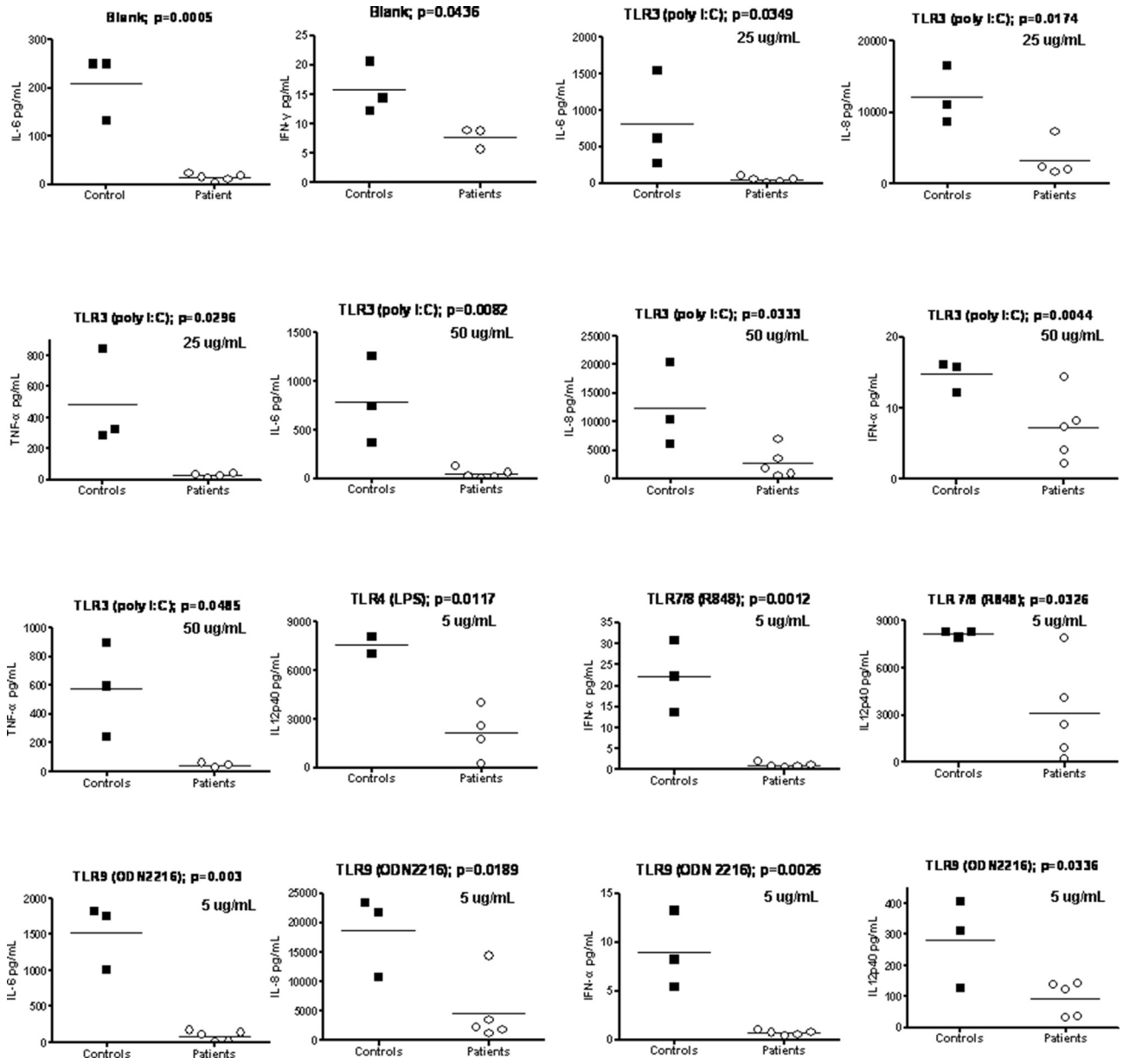

Fig. 4 Significantly lower ($p < 0.05$; unpaired test) scatter diagrams of cytokine production (pg/ml) in blank and through TLR3, TLR4, TLR7/8, and TLR9 signaling in MDDCs from patients with febrile infection-related epilepsy syndrome (FIRES).

In other words, permeability of the blood–brain barrier (BBB) can increase the sympathetic over-activity due to frequent seizures and make lymphocytes and/or some unrecognized anti-neural antibodies influx into the CSF to attack neurons and augment the vicious cycle in the periods of frequent seizure episodes or even status epilepticus [37]. In such conditions, our patients have elevated protein, lymphocytic pleocytosis, or oligoclonal IgG bands in the CSF that are compatible with immune-mediated-like encephalitis. Except down-regulated cytokines of TLR response, our patients share mutual manifestations of febrile infection-related epilepsy syndrome (FIRES), characterized by pharmacoresistant seizures, neither detectable CSF pathogens nor antibodies [38]. Suggested treatments to modulate immune dysregulation and neutralize harmful antibodies include steroids, IVIG, immuno-suppressants, plasmapheresis, and B cell deletion (anti-CD20) regimen [39,40]. After IVIG and methylprednisolone pulse therapy followed by low-dose prednisolone and AEDs, the frequency of seizures was reduced to 2–5 episodes per week.

Notably, TLR4 signaling was grossly intact but significantly decreased IFN-γ and IL12-p40 production implying insufficient IFN-γ-IL12/23 circuits. Such subtle impairment of the IFN-γ-IL12/23 circuit seems not to reach the threshold to develop the phenotype of Mendelian susceptibility to mycobacterial infections (MSMD), presenting with recurrent intracellular salmonella, mycobacterial, varicella and/or opportunistic fungal infections [41].

There are some limitations in this study. First, dissecting peripheral plasmacytoid and myeloid dendritic cells with

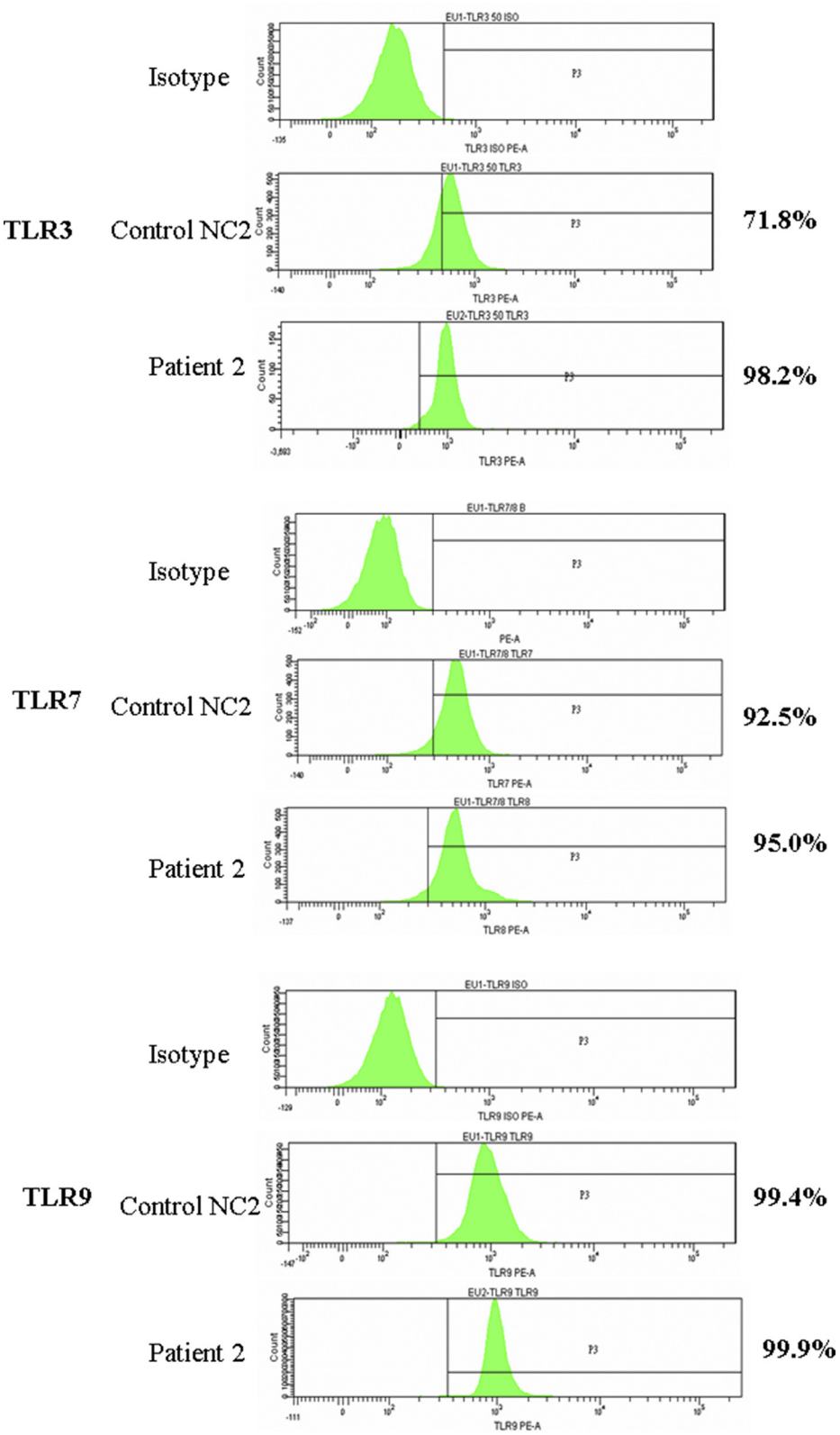

Fig. 5 The intracellular TLR3, TLR7, and TLR9 expressions in the MDDCs of patients with refractory epilepsy and in the controls (representative NC2 with encephalitis and non-refractory epilepsy) were similar.

relatively stronger TLR responses in the peripheral blood, would be more real and representative. However, such subpopulations are extremely few in younger children, and separating these dendritic cells from the peripheral blood to individually evaluate each TLR response would be technically difficult and more laborious. Instead, whole PBMCs were used and MDDCs were grown *in vitro* to ensure consistency. Tissue fibroblasts will be alternative ways to speculate these TLR responses. Second, the patients were prescribed with AEDs to treat seizures. Although the effects of the AEDs on TLR responses in the PBMCs could not completely be omitted, cytokine-grown MDDCs *in vitro* after 5-day-culture may have almost obviated these AEDs effects, if any. Third, in all of the enrolled patients, candidate genes responsible for the defective TLR3 signaling, including at least the coding regions of UNC93B1, TLR3, TRIF, TRAF3 and TBK1 genes [11–19] were wild in spite of similar phenotypes with TRAF3 mutations. Cognate common downstream signaling of TLR3, TLR7/8, TLR9 or/and TLR4, is a logical approach to search for novel and causal molecules by targeting exome sequencing. Lastly, due to the small case numbers enrolled in our study, large-scale studies are still warranted to validate our results.

In conclusion, children with febrile infection-related epilepsy syndrome (FIRES) in this study are found to have impaired TLR3, TLR4, TLR7/8, and TLR9 responses and thus relate to weakened phagocytosis and lower T regulatory cells. Patients with such TLR-related immune impairment may considerably benefit from treatment with immunomodulators such as IVIG and steroid pulse therapy. Further investigations on the mechanisms of effective treatment will be merited.

Conflicts of Interest

The authors have no conflicts of interest relevant to this article.

Funding

Chang Gung Memorial Hospital (Grant CMRPG3B1881 and CMRPG 4B0051-3), the National Science Council (NSC 102-2314-B-182A-039-MY3 and NMRPG3C6071) and Taiwan Foundation for Rare Disorders (TFRD).

Acknowledgements

The authors wish to thank all of the patients and their families for their kind cooperation, as well as their physicians for the referrals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2020.05.007.

REFERENCES

[1] Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, et al. UK Health Protection Agency (HPA) Aetiology of Encephalitis Study Group. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. Lancet Infect Dis 2010;10:835–44.
[2] Ambrose HE, Granerod J, Clewley JP, Davies NWS, Keir G, Cunningham R, et al. UK Aetiology of Encephalitis Study Group. Diagnostic strategy used to establish etiologies of encephalitis in a prospective cohort of patients in England. J Clin Microbiol 2011;49:3576–83.
[3] Glaser CA, Honarmand S, Anderson LJ, Schnurr DP, Forghani B, Cossen CK, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. Clin Infect Dis 2006;43:1565–77.
[4] Sioka C, Fotopoulos A, Kyritsis AP. Paraneoplastic immune-mediated neurological effects of systemic cancers. Expet Rev Clin Immunol 2014;10:621–30.
[5] Vollstedt S, Arnold S, Schwerdel C, Franchini M, Alber G, Di Santo JP, et al. Interplay between alpha/beta and gamma interferons with B, T, and natural killer cells in the defense against herpes simplex virus type 1. J Virol 2004;78:3846–50.
[6] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124:783–801.
[7] Akira S, Takeda K. Toll-like receptor signaling. Nat Rev Immunol 2004;4:499–511.
[8] Kawai T, Akira S. Innate immune recognition of viral infection. Nat Immunol 2006;7:131–7.
[9] Zhang SY, Boisson-Dupuis S, Chapgier A, Yang K, Bustamante J, Puel A, et al. Inborn errors of interferon (IFN)-mediated immunity in humans: insights into the respective roles of IFN-alpha/beta, IFN-gamma, and IFN-lambda in host defense. Immunol Rev 2008;226:29–40.
[10] Casrouge A, Zhang SY, Eidenschenk C, Jouanguy E, Puel A, Yang K, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. Science 2006;314:308–12.
[11] Zhang SY, Jouanguy E, Ugolini S, Smahi A, Elain G, Romero P, et al. TLR3 deficiency in patients with herpes simplex encephalitis. Science 2007;317:1522–7.
[12] Lim HK, Seppänen M, Hautala T, Ciancanelli MJ, Itan Y, Lafaille FG, et al. TLR3 deficiency in herpes simplex encephalitis: high allelic heterogeneity and recurrence risk. Neurology 2014;83:1888–97.
[13] Pérez de Diego R, Mulvey C, Crawford M, Trotter MWB, Lorenzo L, Sancho-Shimizu V, et al. The proteome of Toll-like receptor 3-stimulated human immortalized fibroblasts: implications for susceptibility to herpes simplex virus encephalitis. J Allergy Clin Immunol 2013;131:1157–66.
[14] Zhang SY, Herman M, Ciancanelli MJ, Pérez de Diego R, Sancho-Shimizu V, Abel L, et al. TLR3 immunity to infection in mice and humans. Curr Opin Immunol 2013;25:19–33.
[15] Lafaille FG, Pessach IM, Zhang SY, Ciancanelli MJ, Herman M, Abhyankar A, et al. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. Nature 2012;491:769–73.
[16] Sancho-Shimizu V, Pérez de Diego R, Lorenzo L, Halwani R, Alangari A, Israelsson E, et al. Herpes simplex encephalitis in children with autosomal recessive and dominant TRIF deficiency. J Clin Invest 2011;121:4889–902.
[17] Pérez de Diego R, Sancho-Shimizu V, Lorenzo L, Puel A, Plancoulaine S, Picard C, et al. Human TRAF3 adaptor molecule deficiency leads to impaired Toll-like receptor 3 response and susceptibility to herpes simplex encephalitis. Immunity 2010;33:400–11.

[18] Sancho-Shimizu V, Pérez de Diego R, Lorenzo L, Halwani R, Alangari A, Israelsson E, et al. Herpes simplex encephalitis in children with autosomal recessive and dominant TRIF deficiency. J Clin Invest 2011;121:4889–902.
[19] Herman M, Ciancanelli M, Ou YH, Lorenzo L, Klaudel-Dreszler M, Pauwels E, et al. Heterozygous TBK1 mutations impair TLR3 immunity and underlie herpes simplex encephalitis of childhood. J Exp Med 2012;209:1567–82.
[20] Schreibelt G, Tel J, Sliepen KH, Benitez-Ribas D, Figdor CG, Adema GJ, et al. Toll-like receptor expression and function in human dendritic cell subsets: implications for dendritic cell-based anti-cancer immunotherapy. Cancer Immunol Immunother 2010;59:1573–82.
[21] Sutmuller RP, Morgan ME, Netea MG, Grauer O, Adema GJ. Toll-like receptors on regulatory T cells: expanding immune regulation. Trends Immunol 2006;27:387–93.
[22] Lee WI, Huang JL, Lin SJ, Yeh KW, Chen LC, Ou LS, et al. Applying T-cell receptor excision circles and immunoglobulinκ-deleting recombination excision circles to patients with primary immunodeficiency diseases. Ann Med 2014;46:555–65.
[23] Lee WI, Torgerson TR, Schumacher MJ, Yel L, Zhu Q, Ochs HD. Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. Blood 2005;105:1881–90.
[24] Lee WI, Huang JL, Yeh KW, Yang MJ, Lai MC, Chen LC, et al. Clinical features and genetic analysis of Taiwanese patients with the hyper IgM syndrome phenotype. Pediatr Infect Dis J 2013;32:1010–6.
[25] Lee WI, Kuo ML, Huang JL, Lin SJ, Wu CJ. Distribution and clinical aspects of primary immuno-deficiencies in a Taiwan Pediatric tertiary hospital during a 20-year-period. J Clin Immunol 2005;25:162–73.
[26] Liao SL, Lai SH, Huang JL, Lee NC, Lee WI. Serial cytokine expressions in infants with incontinentia pigmenti. Immunobiology 2013;218:772–9.
[27] Lee WI, Yao TC, Yeh KW, Chen LC, Ou LS, Huang JL; PATCH Study Group. Stronger Toll-like receptor 1/2, 4, and 7/8 but less 9 responses in peripheral blood mononuclear cells in non-infectious exacerbated asthmatic children. Immunobiology 2013;218:192–200.
[28] Berges C, Naujokat C, Tinapp S, Wieczorek H, Höh A, Sadeghi M, et al. A cell line model for the differentiation of human dendritic cells. Biochem Biophys Res Commun 2005;333:896–907.
[29] Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335–76.
[30] Kawai T, Akira S. Pathogen recognition with Toll-like receptors. Curr Opin Immunol 2005;17:338–44.
[31] Korthals M, Safaian N, Kronenwett R, Maihöfer D, Schott M, Papewalis C, et al. Monocyte derived dendritic cells generated by IFN-alpha acquire mature dendritic and natural killer cell properties as shown by gene expression analysis. J Transl Med 2007;5:46.
[32] Yang K, Puel A, Zhang S, Eidenschenk C, Ku CL, Casrouge A, et al. Human TLR-7, -8 and -9 mediated induction of IFN-alpha/beta and -lambda is IRAK-4 dependent and redundant for protective immunity to viruses. Immunity 2005;23:465–78.
[33] Whitley RJ, Roizman B. Herpes simplex virus infections. Lancet 2001;357:1513–8.
[34] Moretti J, Blander JM. Insights into phagocytosis-coupled activation of pattern recognition receptors and inflammasomes. Curr Opin Immunol 2014;26:100–10.
[35] Delogu LG, Deidda S, Delitala G, Manetti R. Infectious diseases and autoimmunity. J Infect Dev Ctries 2011;5:679–87.
[36] Kono DH, Baccala R, Theofilopoulos AN. TLRs and interferons: a central paradigm in autoimmunity. Curr Opin Immunol 2013;25:720–7.
[37] Dalmau J, Tüzün E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007;61:25–36.
[38] Pardo CA, Nabbout R, Galanopoulou AS. Mechanisms of epileptogenesis in pediatric epileptic syndromes: Rasmussen encephalitis, infantile spasms, and febrile infection-related epilepsy syndrome (FIRES). Neurotherapeutics 2014;11:297–310.
[39] Vincent A, Bien CG, Irani SR, Waters P. Autoantibodies associated with diseases of the CNS: new developments and future challenges. Lancet Neurol 2011;10:759–72.
[40] Petit-Pedrol M, Armangue T, Peng X, Bataller L, Cellucci T, Davis R, et al. Encephalitis with refractory seizures, status epilepticus, and antibodies to the GABAA receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. Lancet Neurol 2014;13:276–86.
[41] Lee WI, Huang JL, Wu TS, Lee MH, Chen IJ, Yu KH, et al. Patients with inhibitory and neutralizing auto-antibodies to interferon-γ resemble the sporadic adult-onset phenotype of Mendelian Susceptibility to Mycobacterial Disease (MSMD) lacking Bacille Calmette-Guerin (BCG)-induced diseases. Immunobiology 2013;218:762–71.