Cerebrospinal fluid cyto-/chemokine profile during acute herpes simplex virus induced anti-*N*-methyl-D-aspartate receptor encephalitis and in chronic neurological sequelae

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This article is commented on by Ellul and Griffiths on pages 776–777 of this issue.

PUBLICATION DATA
Accepted for publication 8th February 2017.
Published online 25th April 2017.

ABBREVIATIONS

APRIL	A proliferation-inducing ligand
CSF	Cerebrospinal fluid
CXCL	Chemokine (C-X-C motif) ligand
HSE	Herpes simplex virus encephalitis
HSV	Herpes simplex virus
IVIG	Intravenous immunoglobulins
NMDAR	*N*-methyl-D-aspartate receptor
PCR	Polymerase chain reaction
TNF	Tumour necrosis factor

AIM To examine the cytokine/chemokine profile of cerebrospinal fluid (CSF) during acute herpes simplex virus-induced *N*-methyl-D-aspartate receptor (NMDAR) autoimmunity and in chronic/relapsing post-herpes simplex virus encephalitis (HSE) neurological syndromes.
METHOD We measured longitudinal serial CSF cyto-/chemokines (n=34) and a glial marker (calcium-binding astroglial protein, S100B) in one patient during acute HSE and subsequent anti-NMDAR encephalitis, and compared the results with those from two patients with anti-NMDAR encephalitis without preceding HSE. We also compared cyto-/chemokines in cross-sectional CSF samples from three children with previous HSE who had ongoing chronic or relapsing neurological symptoms (2yr 9 mo–16y after HSE) with those in a group of children having non-inflammatory neurological conditions (n=20).
RESULTS Acute HSE showed elevation of a broad range of all T-helper-subset-related cyto-/chemokines and S100B whereas the post-HSE anti-NMDAR encephalitis phase showed persistent elevation of two of five T-helper-1 (chemokine [C-X-C motif] ligand 9 [CXCL9], CXCL10), three of five predominantly B-cell (CXCL13, CCL19, a proliferation-inducing ligand [APRIL])-mediated cyto-/chemokines, and interferon-α. The post-HSE anti-NMDAR encephalitis inflammatory response was more pronounced than anti-NMDAR encephalitis. All three chronic post-HSE cases showed persistent elevation of CXCL9, CXCL10, and interferon-α, and there was histopathological evidence of chronic lymphocytic inflammation in one biopsied case 7 years after HSE. Two of three chronic cases showed a modest response to immune therapy.
INTERPRETATION HSE-induced anti-NMDAR encephalitis is a complex and pronounced inflammatory syndrome. There is persistent CSF upregulation of cyto-/chemokines in chronic or relapsing post-HSE neurological symptoms, which may be modifiable with immune therapy. The elevated cyto-/chemokines may be targets of monoclonal therapies.

Post-herpes simplex virus encephalitis (post-HSE) relapses have been reported in 25% to 35% of children and approximately 12% of adults, despite adequate anti-viral treatment, and can cause chronic relapsing or progressive neurological symptoms.[1–3] Some of these relapsing patients develop *N*-methyl-D-aspartate receptor (NMDAR) antibodies, some develop unknown cell-surface antibodies, whereas others show no evidence of cell surface antibodies.[4] Evidence of an intrathecal synthesis of herpes simplex virus (HSV)-specific immunoglobulin-G, oligoclonal bands, and neopterin[5,6] years after acute infection supports a persistent intrathecal immune activation in some patients with previous HSE. Improved understanding of the specific immune pathogenesis could provide novel therapies in the future. For example, tocilizumab (an interleukin-6 blocker) has been shown to be useful in patients with autoimmune encephalitis who are refractory to rituximab.[7]

Cytokines and chemokines are pleiotropic biologically active polypeptide molecules that help with immune cell activation, recruitment, and differentiation at sites of inflammation. Previous studies have reported elevation of some cerebrospinal fluid (CSF) cyto-/chemokines (interferon-γ, interleukin-6, chemokine [C-X-C motif] ligand 13 [CXCL13], B-cell activating factor [BAFF], a proliferation-inducing

 DOI: 10.1111/dmcn.13431

ligand [APRIL]) in acute HSE.[5,8–10] The CSF protein biomarkers of neuroaxonal and glial cell injury including S100B are elevated in acute HSE, which may indicate the extent of neural injury.[10,11]

The literature on CSF cyto-/chemokines and markers of neuronal injury in post-HSE autoimmune encephalitis is limited.[5,12] Here we report the longitudinal CSF cyto-/chemokine and S100B profile during transition from the acute infective phase of HSE to the post-HSE autoimmune (anti-NMDAR) encephalitis phase, and provide evidence for persistent cyto-/chemokine elevation in chronic post-HSE neurological syndromes many years after initial HSE illness.

What this paper adds

- Inflammatory response is higher in anti-*N*-methyl-D-aspartate receptor (anti-NMDAR) encephalitis if is it preceded by herpes simplex encephalitis (HSE).
- Aggressive immune treatment in acute post-HSE anti-NMDAR illness improves the chances of a good outcome.
- Children with previous HSE and ongoing neurological symptoms may have persistent immune activation and chronic encephalitis.
- Persistent cerebrospinal fluid upregulation of cyto-/chemokines may be modifiable with immune therapy.

METHOD

As part of the ethically approved protocol (LNRSSA/14/SCHN/283), we wrote to all parents and gained written consent for CSF cytokine study to use stored acute CSF samples, and publication of the results. In addition, we obtained written consent from the parents to present histopathology finding and videos.

Patients and comparison group characteristics

Serial CSF cytokine and chemokine analysis was performed in one patient with HSE followed by NMDAR encephalitis (acute post-HSE NMDAR), and compared with two cases with anti-NMDAR encephalitis without preceding HSV infection (anti-NMDAR cases 1 and 2). In addition, three children with previous HSE who had ongoing chronic or relapsing neurological symptoms (chronic post-HSE cases 1–3) had CSF sampled for routine and cytokine analysis 2 years and 9 months to 16 years after HSE, and were compared with those in a group of children having non-inflammatory neurological conditions (n=20, detailed in Appendix S1, online supporting information). The median age of the comparison group at the time of CSF sampling was 4 years 11 months (range 4mo–14y).

Multiplex cyto-/chemokine and S100B immunoassay

All CSF samples were collected during routine diagnostic work-up and were frozen at −40°C until analysis. Thirty-two cytokines and S100B were measured by multiplexed fluorescent bead-based immunoassay detection (MILLIPLEX MAP system, Millipore Corporation, MO, USA) according to the manufacturer's instructions, using a combination of 23-plex (MPHCYTOMAG60K23), 6-plex (MPHCYP2-MAG62K06), 3-plex (MPHCYP3MAG63K03), and 1-plex kits (MPHNDG4MAG68K01). Cytokine concentrations were calculated by reference to a standard curve for each one. APRIL and BAFF were performed using ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).[13]

Histopathology

Histopathological examination of the temporal lobe biopsy specimen in chronic post-HSE case 3 was performed using conventional methods and immunostaining with Bond 3 Immunohistochemical Stainer. Demonstration of bound antibody was achieved using a Bond Polymer Refine Peroxidase system (DS9800). DAB (3,3′-diaminobenzidine) was used as a chromogen with haematoxylin as the counterstain. The antibodies and clones used were CD4 (4B12), CD8 (4B11), CD20 (L26), CD68 (PG-M1), and CD163 (MRQ26).

RESULTS

Longitudinal CSF testing in acute post-HSE anti-NMDAR encephalitis

Acute post-HSE-NMDAR clinical case

A 13-month-old female presented in 2014 initially with fever for 5 days, progressive lethargy, and left focal seizures with left-sided weakness. Her CSF analysis revealed pleocytosis (seven polymorphs, 202 monocytes, and 424 red blood cells, protein 0.85mg/dL), elevated neopterin (573nmol/dL, normal <30), and positive HSV polymerase chain reaction (PCR). At this stage, serum and CSF NMDAR antibody were negative. Magnetic resonance imaging (MRI) showed high signal in the right temporal, frontal lobe, and insular regions (Fig. 1). She was treated with intravenous acyclovir and phenytoin. She was admitted to an intensive care unit with focal status epilepticus and received one dose of methylprednisolone on day 15.

After a brief period of improvement in alertness, from day 19 she developed progressive irritability, autonomic disturbances in the form of tachycardia, low-grade fever, and insomnia. On day 31, she developed severe dyskinesias in the form of tongue thrusting, loud vocalization, and fast, continuous, fairly symmetrical large-amplitude choreoathetoid movements of her limbs, trunk and neck that were treated with infusions of midazolam, clonidine and dexmetedomidine, trimeprazine, and chloral hydrate in a paediatric intensive care unit (Video S1, online supporting information). Her repeat CSF analysis performed during clinical relapse on day 33 of illness showed positive NMDAR antibodies but was negative for HSV PCR, as shown in Figure 1. Her CSF pleocytosis and neopterin continued to show decline during the clinical relapse. Her repeat MRI on day 38 showed worsening high signal in the right temporal and occipital white matter without any new areas of necrosis (Fig. 1). She was treated with methylprednisolone (30mg/kg/day for 3d), intravenous immunoglobulins (IVIG, 2g/kg) on day 33, and rituximab (weekly doses of 375mg/m^2 for 4 wks) on day 43. She started showing signs of improvement in the form of improved encephalopathy a week after the first dose of

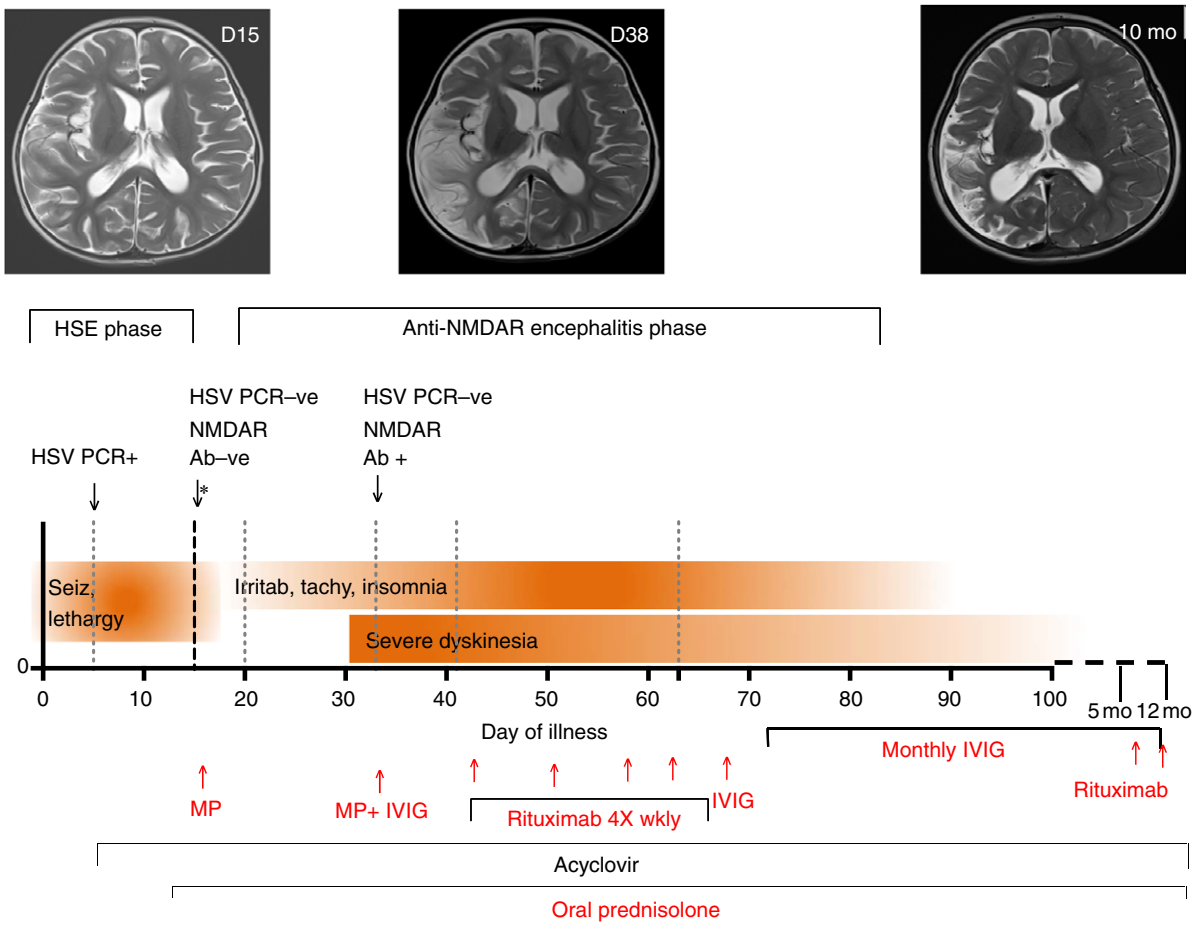
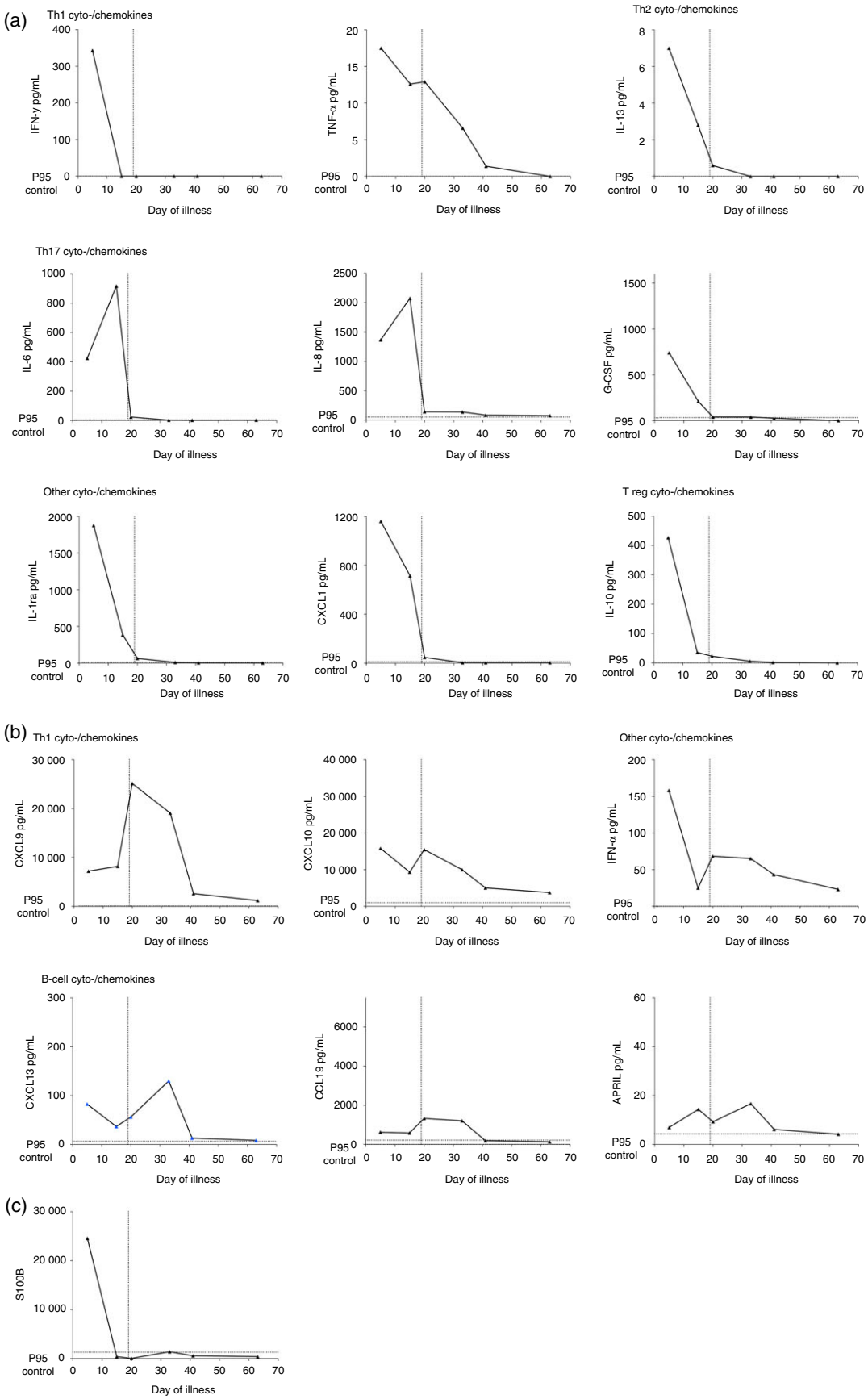


Figure 1: The clinical, cerebrospinal fluid (CSF) timing from the onset of illness, magnetic resonance imaging (MRI) and treatment details of patient with acute post-herpes simplex virus encephalitis (HSE) anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis. Serial axial T2-weighted magnetic resonance images of brain show (a) hyperintense signal involving the right temporal, frontal and insular cortex in acute HSE phase on day 15 (D15); (b) increased signal in right temporal and occipital lobe white matter during anti-NMDAR illness phase on day 38 (D38); and (c) resolution of white matter signal and residual extensive right temporal lobe encephalomalacia at 10 months (10mo) follow-up. *Vertical thick dotted line represents the timing when herpes simplex virus (HSV) polymerase chain reaction (PCR) became negative in acute post-HSE anti-NMDAR encephalitis. Vertical thin dotted line represents the time of CSF sampling from onset of illness. Seiz, seizures; Irritab, irritability; tachy, tachycardia; MP, methylprednisolone; IVIG, intravenous immunoglobulins; Ab, antibody. [Colour figure can be viewed at wileyonlinelibrary.com].

rituximab. She underwent serial CSF studies to monitor inflammation using CSF neopterin (requested urgently) and to escalate immunomodulatory treatment (on days 5, 15, 20, 33, 41, and 63 from onset of illness; Appendix S1). Residual CSF was used for cytokine research studies, after written parental consent. At 5 and 12 months' follow-up, she was given second and third doses of rituximab owing to repopulation of B cells and continued on monthly IVIG. At the age of 2 years 8 months (18mo follow-up), she remained B-cell depleted

Figure 2: Serial cerebrospinal fluid (CSF) cyto-/chemokine and S100B profile during transition from acute herpes simplex virus encephalitis (HSE) to post-HSE anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis. (a) Cyto-/chemokines that were elevated in HSE phase that declined during anti-NMDAR encephalitis phase are presented. (b) Cyto-/chemokines that showed secondary elevation in acute post-HSE anti-NMDAR encephalitis phase. Two of five T-helper-1-related chemokines (chemokine [C-X-C motif] ligand 10 [CXCL10], CXCL9), three of the five B-cell cyto-/chemokines (CXCL13, CCL19, a proliferation-inducing ligand [APRIL]), and interferon-α (interferon-α) showed secondary elevation during the acute post-HSE anti-NMDAR encephalitis phase. (c) S100B was only elevated in the HSE phase, and rapidly declined. (For full cytokine data and comparison with two anti-NMDAR encephalitis patients, see Fig. S1.) Vertical dotted line on day 19 represents clinical onset of symptoms of the anti-NMDAR encephalitis phase. The 95th centile of the 20 non-inflammatory controls is presented (P95).


(a)
Th1 cyto-/chemokines
IFN-γ pg/mL
TNF-α pg/mL
Th2 cyto-/chemokines
IL-13 pg/mL
Th17 cyto-/chemokines
IL-6 pg/mL
IL-8 pg/mL
G-CSF pg/mL
Other cyto-/chemokines
IL-1ra pg/mL
CXCL1 pg/mL
T reg cyto-/chemokines
IL-10 pg/mL
(b)
Th1 cyto-/chemokines
CXCL9 pg/mL
CXCL10 pg/mL
Other cyto-/chemokines
IFN-α pg/mL
B-cell cyto-/chemokines
CXCL13 pg/mL
CCL19 pg/mL
APRIL pg/mL
(c)
S100B
P95
control
Day of illness

(last rituximab at 12mo of disease), remained on oral prednisolone (1mg alternate days) and acyclovir, and was making good progress in development, except for mild speech delay (see Video S1). The plan was to allow repopulation of B cells without re-dosing rituximab and to monitor closely, clinically, and using CSF monitoring (CSF neopterin). She remained on monthly IVIG for a minimum of 6 further months.

The clinical course, treatment details, and timing of CSF sampling of the two anti-NMDAR encephalitis cases (without HSE) are presented in Appendix S1.

Longitudinal CSF cyto-/chemokines in HSE (infective) and post-HSE anti-NMDAR encephalitis (autoimmune phase)

The cyto-/chemokine data are grouped on the basis of the predominant T-cell effector pathway involved. A broad profile of proinflammatory cyto-/chemokines including T-helper-1-related cyto-/chemokines (tumour necrosis factor-α [TNF-α], interferon-γ), T-helper-2 (interleukin-13), Treg (interleukin-10), and others (GCSF, CXCL1, and interleukin-1-ra) was significantly elevated during the HSV PCR-positive infective phase of the illness and declined subsequently after day 15, despite clinical relapse in the anti-NMDAR encephalitis phase (autoimmune) (Fig. 2a). Some T-helper-17 cytokines (interleukin-6 and interleukin-8) showed transient elevation at day 15 before the onset of symptoms associated with anti-NMDAR encephalitis. Some cytokines became secondarily elevated during the relapse associated with production of NMDAR antibodies. Two of five T-helper-1-related chemokines (CXCL10, CXCL9), three of the five B-cell cyto-/chemokines (CXCL13, CCL19, and APRIL), and interferon-α were elevated in the anti-NMDAR encephalitis phase (Fig. 2b). Patients with anti-NMDAR encephalitis (anti-NMDAR cases 1 and 2 without preceding HSE) showed similar but less pronounced elevation of T-helper-1 (CXCL10, TNF-α), B-cell (CXCL13, CCL19) cyto-/chemokines, and interferon-α. (All cytokines in the post-HSE-NMDAR case and the two anti-NMDAR comparison cases are presented in Figure S1, online supporting information).

S100B

S100B was markedly elevated only in the acute HSE phase of encephalitis, and the levels did not increase in the anti-NMDAR encephalitis phase despite clinical symptoms (Fig. 2c).

Latent cross-sectional CSF testing in chronic post-HSE cases

Clinical cases

Chronic post-HSE case 1. A 4-year-old male presented with HSE (CSF HSV PCR-positive) followed by relapse (CSF NMDAR antibody-positive) in the form of choreoathetoid movements on day 15 (previously described by Mohammad et al.[14]), and responded to immune treatment with methylprednisolone, cyclophosphamide, and oral valacyclovir. He deteriorated twice, associated with febrile illnesses (15mo and 2y 6mo after initial HSE illness) in the form of fluctuating developmental regression, behaviour symptoms, increasing dyskinesias, and altered sleep patterns. His repeat CSF 3 months after his second deterioration (2y 9mo after initial HSE) showed high neopterin (85.6nmol/dL), positive oligoclonal bands, and was used for the cyto-/chemokine testing in our study. During both deteriorations he was treated with methylprednisolone, oral steroids, IVIG, and rituximab, which led to partial improvement in behavioural outbursts and dyskinesias. At the last follow-up at the age of 7 years, he continued to have moderate to severe cognitive problems and had limited language.

Chronic post-HSE case 2. A 10-month-old female presented in December 2002 with fever, lethargy, and focal seizures and was diagnosed to have HSE on the basis of CSF studies, positive CSF HSV PCR, and MRI evidence of T2-weighted hyperintense signal change in the right temporal lobe. She was treated with intravenous acyclovir for 2 weeks and phenytoin with a good recovery. At the age of 22 months (12mo after HSE illness), towards the end of 2003, she showed signs of deterioration with multiple types of seizure refractory to anticonvulsant medication and regression in developmental milestones. She did not have any dyskinesia or movement disorder. Her serum and CSF during acute relapse were not available for NMDAR antibody testing. She had a fluctuating clinical course with prolonged periods of encephalopathy in a vegetative stage with/without increased seizures interspersed by episodes when she was able to walk, play with toys with limited concentration, and attend home school. Her CSF at the age of 11 years showed no cells, elevated neopterin (31.8nmol/dL), positive intrathecal oligoclonal bands, negative NMDAR antibodies, negative HSV PCR, and was used for the cyto-/chemokine testing in our study. In view of persistent intrathecal inflammation, she was started on monthly courses of intravenous methylprednisolone (1g×3d) and IVIG (2g/kg) for 6 months, to which she showed short-lived but definite decrease in seizure frequency, more seizure-free days, and overall improved levels of alertness and purposeful interaction. At the last follow-up at 14 years (12y 7mo after HSE illness) she could walk independently but had severe cognitive and language impairment. It was decided to continue monthly-pulsed methylprednisolone and IVIG for the next 6 to 12 months.

Chronic post-HSE case 3. A 12-month-old female presented in 1995 with drowsiness, irritability, and focal seizures. Her CSF showed pleocytosis (6 mono$\times 10^6$/L, 8 RBC$\times 10^6$/L), positive HSV PCR, and brain MRI showed T2-weighted hyperintensity in the left temporal lobe. Despite treatment with acyclovir, she deteriorated 5 weeks after discharge with irritability and dyskinesias, and was treated with 3 weeks of intravenous acyclovir, intravenous methylprednisolone, and oral prednisolone. Serum taken during the episode of chorea (tested in retrospect in 2009) showed elevated autoantibodies against dopamine-2 receptor, but it was negative for antibodies to NMDAR. After a good recovery for 12 months after initial HSE, at the age

of 2 years 6 months, she deteriorated and continued to have a fluctuating course with cognitive decline and refractory seizures resistant to multiple antiepileptic drugs. She underwent left temporal lobectomy at the age of 7 years. CSF analysis at the age of 17 years, 16 years after HSE, showed no cells, elevated protein (0.51mg/dL), mildly elevated neopterin (29.3nmol/dL), was negative for oligoclonal bands, and was used for the cyto-/chemokine testing in our study. The details of this case have been previously described.[14]

CSF cyto-/chemokine elevation in chronic post-HSE cases

The T-helper-1-related chemokines (CSF CXCL9, CXCL10) and interferon-α were elevated in all three cases of chronic post-HSE neurological syndromes compared with those in the group having non-inflammatory neurological conditions (Fig. 3). In addition to these cyto-/chemokines, the B-cell-related chemokine CXCL13 was also elevated in chronic post-HSE cases 1 and 2. In chronic post-HSE case 3, there was more widespread elevation of T-helper-1 (interferon-γ, CXCL10, TNF-α), T-helper-2 (CCL11, interleukin-13), T reg (interleukin-10), T-helper-17 (interleukin-23, interleukin-17A), and other cyto-/chemokines (interferon-α, CXCL1, interleukin-1b, and interleukin-1ra) whereas B-cell-related cyto-/chemokines were normal (Fig. S2, online supporting information).

The S100B showed minimal elevation only in chronic post-HSE case 1 (Fig. S2).

Histopathology

Chronic post-HSE case 3 underwent left temporal lobectomy 7 years after initial HSE illness because of refractory focal epilepsy. Histopathological examination of the temporal lobectomy specimen showed a patchy cortical, perivascular, and leptomeningeal infiltrate of lymphocytes, macrophages, gliosis, areas of cavitation, and foci of mineralization. Immunostaining revealed a pronounced chronic inflammatory infiltrate of $CD3^+$, $CD8^+$, occasional $CD4^+$ T and $CD20^+$ B lymphocytes, and a patchy infiltrate of macrophages containing ($CD163^+$>$CD68^+$) (Fig. 4). HSV viral DNA was demonstrated by PCR despite being negative on immunoperoxidase method.

DISCUSSION

The acute post-HSE NMDAR case had the characteristic biphasic clinical course and responded well to sustained immunosuppressive treatment, as previously described.[2,4,14] The absence of NMDAR antibodies in the initial CSF sample during HSE and the appearance of CSF NMDAR antibodies at clinical relapse demonstrates the infection-driven autoantibody production, and highlights interdependent mechanisms between infection and autoimmunity. The elevation of a broad range of cyto-/chemokines including interferon-γ was noted in the acute HSE phase, which declined subsequently during the anti-NMDAR encephalitis phase. The rapid decline of cyto-/chemokines (interleukin-6, interleukin-8, TNF-α, interleukin-13,

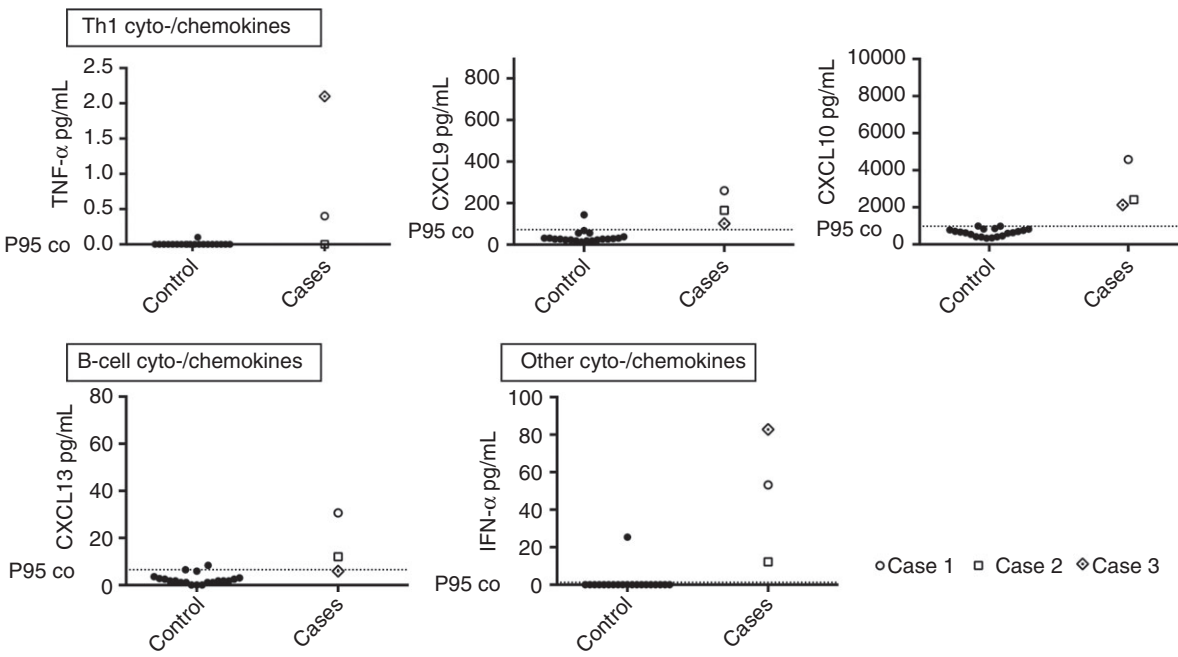


Figure 3: Elevated cerebrospinal fluid cyto-/chemokines in chronic post-herpes simplex virus encephalitis (post-HSE) cases according to T- and B-cell effector cells. The data are compared with 20 non-inflammatory controls (95th centile shown). All three chronic post-HSE cases showed persistent elevation of chemokine (C-X-C motif) ligand 9 (CXCL9), CXCL10, and interferon-α (interferon-α) (2y 9 mo–16y after initial HSE illness). Only cyto-/chemokines that were elevated in a minimum of two patients are presented. TNF, tumour necrosis factor; co, control.

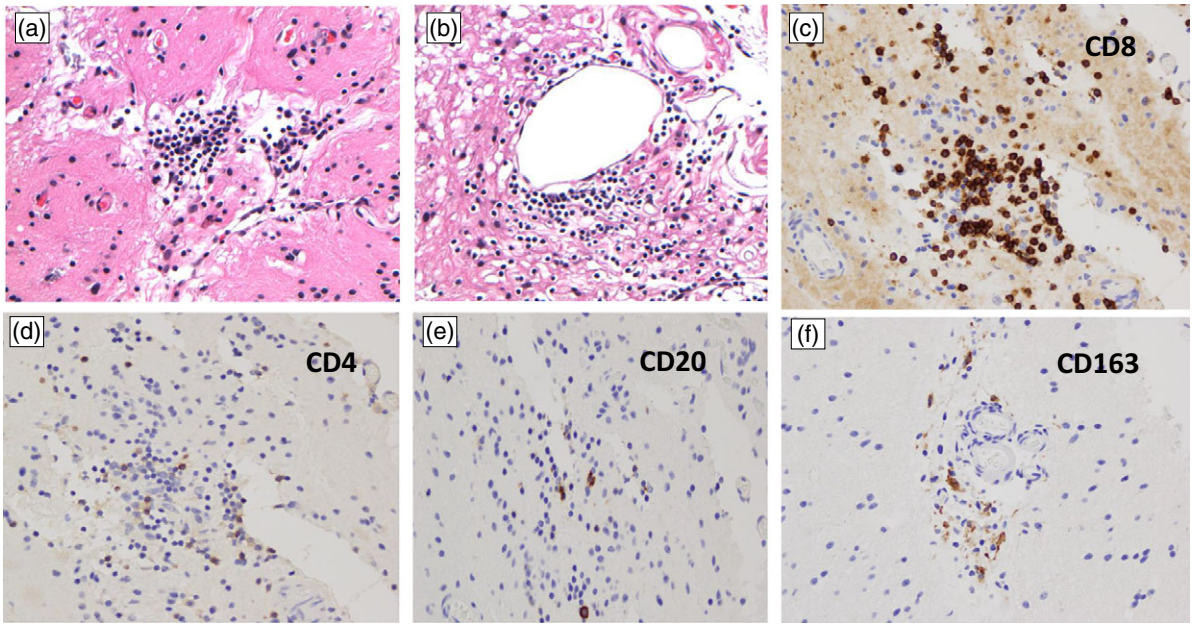


Figure 4: Histopathology of temporal lobe specimen of chronic post-herpes simplex virus encephalitis (post-HSE) case 3 (7y after previous HSE) illustrates persistent infiltrates of (a) diffuse inflammatory cells in parenchyma and (b) perivascular space, (c) $CD8^+$ T lymphocytes, (d) occasional $CD4^+$ T lymphocytes, (e) occasional $CD20^+$ B lymphocytes, and (f) activated macrophages (CD 163^+). [Colour figure can be viewed at wileyonlinelibrary.com].

G-CSF, interleukin-10) (Fig. 2) after the initial rise in our index case could be partly related to intravenous acyclovir use and steroid treatment.[8] A secondary elevation of some of T-helper-1 (CXCL9, CXCL10) and B-cell-associated cyto-/chemokines (CXCL13, CCL19, APRIL) and interferon-α in the anti-NMDAR encephalitis (autoimmune) phase emphasizes the ongoing inflammation and complex immune mechanisms in acute post-HSE anti-NMDAR encephalitis. Similar to our study, previous studies on serial changes in intrathecal cytokines and chemokines in patients with HSVE showed a decline in interferon-γ, neopterin, and interleukin-6 levels after the acute phase of HSE.[5,15]

Interferon-γ is secreted in response to HSV-1 during primary infection, and helps to control viral replication by promoting natural killer- (NK) and T-cell-specific responses, and production of interferon-γ-mediated chemokines.[16] Among elevated cyto-/chemokines in the acute post-HSE anti-NMDAR encephalitis phase, CXCL9 and CXCL10 help with trafficking of activated $CD4^+$, $CD8^+$ T cells and NK cells,[17] whereas CXCL13, CCL19, and APRIL help in B-cell recruitment, proliferation, clonal expansion, and thereby promote antibody synthesis causing immune-mediated brain injury.[18] Interferon-α plays an important role in inhibiting viral replication and induces a positive feedforward loop in the development and activation of dendritic, T-helper-1, NK, and B-cell production of cyto-/chemokines.[16] The overall inflammatory response was less pronounced in the anti-NMDAR encephalitis patients without HSE (shown in Appendix S1).[19,20] Elevated CSF S100B reflects the acute destruction of astroglial cell bodies in the early phase of HSE, and the lack of elevation of S100B in the anti-NMDAR encephalitis phase argues against direct viral cytotoxicity as a major pathogenic mechanism.[10,11]

There are reports describing intractable seizures, slowly progressive hemiparesis, and progressive cognitive and behavioural deterioration for many years after herpes simplex encephalitis in children and adults owing to ongoing inflammation[1,14] or, rarely, reactivation of virus.[3,21] Persistent elevation of cyto-/chemokines (CXCL9, CXCL10, interferon-α) observed in the three patients with chronic fluctuating symptoms in our study supports ongoing intrathecal immune activation many years (2yr 9 mo-16y) after initial HSE.[22,23] The immunopathogenesis may be heterogenous in post-HSE relapsing/chronic symptoms, as reflected by the variability in antibody association and cyto-/chemokine profile in our three chronic HSE patients.[4,5] In our series, only one of the three chronic post-HSE cases had proven anti-NMDAR antibodies whereas the other two were negative. Chronic post-HSE cases 1 and 2 showed oligoclonal band positivity and elevation of B-cell-related cyto-/chemokines, whereas chronic post-HSE case 3 had predominant elevation of T-helper-1-, T-helper-2-, and T-helper-17-related cyto-/chemokines and lacked B-cell-related cyto-/chemokines, which was further supported by prominent infiltration of $CD8^+$ T lymphocytes as noted on histopathology 7 years after HSE illness (Fig. 4).[3,5,17] In chronic post-HSE case 3, CSF testing 16 years after acute HSE was negative for CSF HSV PCR, but HSV DNA was evident in the histopathology specimen despite negative immune histochemistry. It is possible that periodic, subclinical reactivations of a latent persistent virus

and intermittent antigen expression may cause dysregulation of memory CD8 T-cell homeostasis, immune activation, and cytokine expression.[22,24]

Previous studies reported that patients with HSE treated with steroids had a better outcome and a rapid decline of cyto-/chemokines.[8,25,26] Similarly, in our acute post-HSE NMDAR case who had longitudinal CSF cytokine analysis, aggressive immunomodulatory treatment with induction and ongoing rituximab, IVIG, and steroids resulted in clinical improvements, and the patient remained well without relapses 18 months after HSE except for mild speech delay. By contrast, the chronic post-HSE cases 2 and 3 were treated latently (many years later) with courses of methylprednisolone, IVIG, and valacyclovir, which resulted in some subjective but marginal improvements. It is unclear whether aggressive early immune therapy can improve the long-term outcomes, and whether these patients need chronic aggressive immune suppression, as is true for severe chronic autoimmune disorders. Monoclonal antibodies against cytokines and chemokines hold promise in controlling the inflammatory response. The elevation of pathogenic cyto-/chemokines such as CSF CXCL10 and CXCL13 may suggest a potential role for these molecules in disease, and possible targets for immunomodulatory therapies. However, as these cyto-/chemokines are elevated in multiple central nervous system (CNS) inflammatory disorders, including a variety of CNS infections,[9,27] further work is required to understand pro- and anti-inflammatory effects of these molecules during the disease course of different inflammatory diseases of the CNS, and to develop strategies to control inflammation.[28,29]

The limitation of this study includes the small sample size, the heterogeneous nature of the clinical cases, and the lack of standardization of timing of CSF sampling given the retrospective nature of the report. However, the findings from this study support the involvement of complex immune mechanisms during acute post-HSE relapse and chronic post-HSE neurological syndromes. CSF cyto-/chemokines may be useful biomarkers of immune activation, although the nature of this inflammation (autoimmune or other inflammation) is unlikely to be diagnosed with cyto-/chemokines alone, and may require other serum/CSF biomarkers or even biopsy in the appropriate setting. A greater understanding of the contribution of cyto-/chemokines within the CNS in response to HSV-1 invasion is necessary to identify candidate molecules as targets for therapeutic intervention to reduce inflammation.

ACKNOWLEDGEMENTS

This work was supported by a scholarship from the Petre Foundation and an Australian Postgraduate award. The authors have stated that they had no interests which might be perceived as posing a conflict or bias.

SUPPORTING INFORMATION

The following additional material may be found online:

Appendix S1: Clinical cases of autoimmune encephalitis included in acute longitudinal cerebrospinal fluid (CSF) testing.

Video S1: Video of acute post-HSE NMDAR encephalitis pre- and posttreatment showing clinical improvement in response to immune therapy.

Figure S1: Serial CSF cyto-/chemokine and S100B profile during transition from acute herpes simplex virus encephalitis (HSE) to post-HSE *N*-methyl-D-aspartate receptor (NMDAR) encephalitis, and comparison with anti-NMDAR encephalitis without preceding HSE. These cyto-/chemokines are higher in the acute post-HSE anti-NMDAR case (blue line) compared to the anti-NMDAR encephalitis cases (red and green). S100B is not elevated in acute post-HSE anti-NMDAR encephalitis phase and in the two patients with anti-NMDAR encephalitis. The 95th centile of the 20 non-inflammatory controls is presented (P95). The *x*–*y* axis intersection represents D 19, which is the day of onset of clinical symptoms of anti-NMDAR encephalitis phase.

Figure S2: Complete CSF cyto-/chemokine profile and S100B in chronic post-HSE cases according to T- and B-cell effector cells. The data is compared to 20 non-inflammatory controls (95th centile shown).

REFERENCES

1. Hacohen Y, Deiva K, Pettingill P, et al. *N*-methyl-*D*-aspartate receptor antibodies in post-herpes simplex virus encephalitis neurological relapse. *Mov Disord* 2014; **29**: 90–6.
2. Titulaer MJ, Leypoldt F, Dalmau J. Antibodies to *N*-methyl-*D*-aspartate and other synaptic receptors in choreoathetosis and relapsing symptoms post-herpes virus encephalitis. *Mov Disord* 2014; **29**: 3–6.
3. Jay V, Hwang P, Hoffman HJ, Becker LE, Zielenska M. Intractable seizure disorder associated with chronic herpes infection. HSV1 detection in tissue by the polymerase chain reaction. *Childs Nerv Syst* 1998; **14**: 15–20.
4. Armangue T, Leypoldt F, Malaga I, et al. Herpes simplex virus encephalitis is a trigger of brain autoimmunity. *Ann Neurol* 2014; **75**: 317–23.
5. Aurelius E, Andersson B, Forsgren M, Skoldenberg B, Strannegard O. Cytokines and other markers of intrathecal immune response in patients with herpes simplex encephalitis. *J Infectious Dis* 1994; **170**: 678–81.
6. Armangue T, Moris G, Cantarin-Extremera V, et al. Autoimmune post-herpes simplex encephalitis of adults and teenagers. *Neurology* 2015; **85**: 1736–43.
7. Lee WJ, Lee ST, Moon J, et al. Tocilizumab in autoimmune encephalitis refractory to rituximab: an institutional cohort study. *Neurotherapeutics* 2016; **13**: 824–32.
8. Kamei S, Taira N, Ishihara M, et al. Prognostic value of cerebrospinal fluid cytokine changes in herpes simplex virus encephalitis. *Cytokine* 2009; **46**: 187–93.
9. Hytonen J, Kortela E, Waris M, Puustinen J, Salo J, Oksi J. CXCL13 and neopterin concentrations in cerebrospinal fluid of patients with Lyme neuroborreliosis and other diseases that cause neuroinflammation. *J Neuroinflamm* 2014; **11**: 103.
10. Skoldenberg B, Aurelius E, Hjalmarsson A, et al. Incidence and pathogenesis of clinical relapse after herpes simplex encephalitis in adults. *J Neurol* 2006; **253**: 163–70.
11. Studahl M, Rosengren L, Gunther G, Hagberg L. Difference in pathogenesis between herpes simplex virus type 1 encephalitis and tick-borne encephalitis demonstrated by means of cerebrospinal fluid markers of glial and neuronal destruction. *J Neurol* 2000; **247**: 636–42.
12. Ygberg S, Fowler A, Wickstrom R. Cytokine and chemokine expression in CSF may differentiate viral and autoimmune NMDAR Encephalitis in children. *J Child Neurol* 2016; **31**: 1450–6.

13. Kothur K, Wienholt L, Tantsis EM, et al. B Cell, Th17, and neutrophil related cerebrospinal fluid cytokine/chemokines are elevated in MOG antibody associated demyelination. *PLoS ONE* 2016; **11**: e0149411.
14. Mohammad SS, Sinclair K, Pillai S, et al. Herpes simplex encephalitis relapse with chorea is associated with autoantibodies to *N*-methyl-*D*-aspartate receptor or dopamine-2 receptor. *Mov Disord* 2014; **29**: 117–22.
15. Lebon P, Boutin B, Dulac O, Ponsot G, Arthuis M. Interferon gamma in acute and subacute encephalitis. *BMJ* 1988; **296**: 9–11.
16. Vollstedt S, Arnold S, Schwerdel C, 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.
17. Wuest TR, Carr DJ. Dysregulation of CXCR3 signaling due to CXCL10 deficiency impairs the antiviral response to herpes simplex virus 1 infection. *J Immunol* 2008; **181**: 7985–93.
18. Brandes M, Legler DF, Spoerri B, Schaerli P, Moser B. Activation-dependent modulation of B lymphocyte migration to chemokines. *Int Immunol* 2000; **12**: 1285–92.
19. Leypoldt F, Hoftberger R, Titulaer MJ, et al. Investigations on CXCL13 in anti-N-methyl-D-aspartate receptor encephalitis: a potential biomarker of treatment response. *JAMA Neurol* 2015; **72**: 180–6.
20. Liba Z, Kayserova J, Elisak M, et al. Anti-N-methyl-D-aspartate receptor encephalitis: the clinical course in light of the chemokine and cytokine levels in cerebrospinal fluid. *J Neuroinflamm* 2016; **13**: 55.
21. Yamada S, Kameyama T, Nagaya S, Hashizume Y, Yoshida M. Relapsing herpes simplex encephalitis: pathological confirmation of viral reactivation. *J Neurol Neurosurg Psychiatry* 2003; **74**: 262–4.
22. Steiner I. Herpes simplex virus encephalitis: new infection or reactivation? *Curr Opin Neurol* 2011; **24**: 268–74.
23. Sellner J, Dvorak F, Zhou Y, et al. Acute and long-term alteration of chemokine mRNA expression after antiviral and anti-inflammatory treatment in herpes simplex virus encephalitis. *Neurosci Lett* 2005; **374**: 197–202.
24. Lang A, Brien JD, Nikolich-Zugich J. Inflation and long-term maintenance of CD8 T cells responding to a latent herpesvirus depend upon establishment of latency and presence of viral antigens. *J Immunol* 2009; **183**: 8077–87.
25. Kamei S, Sekizawa T, Shiota H, et al. Evaluation of combination therapy using aciclovir and corticosteroid in adult patients with herpes simplex virus encephalitis. *J Neurol Neurosurg Psychiatry* 2005; **76**: 1544–9.
26. Ramakrishna C, Openshaw H, Cantin EM. The case for immunomodulatory approaches in treating HSV encephalitis. *Future Virol* 2013; **8**: 259–72.
27. Wang SM, Lei HY, Yu CK, Wang JR, Su IJ, Liu CC. Acute chemokine response in the blood and cerebrospinal fluid of children with enterovirus 71-associated brainstem encephalitis. *J Infect Dis* 2008; **198**: 1002–6.
28. Carr DJ, Chodosh J, Ash J, Lane TE. Effect of anti-CXCL10 monoclonal antibody on herpes simplex virus type 1 keratitis and retinal infection. *J Virol* 2003; **77**: 10037–46.
29. Klimatcheva E, Pandina T, Reilly C, et al. CXCL13 antibody for the treatment of autoimmune disorders. *BMC Immunol* 2015; **16**: 6.