Intravenous Immunoglobulin in Pediatric Neuropsychiatric Lupus Triggered by Epstein-Barr Virus Cerebral Infection

Claudia Brogna MD1,4*, Raffaele Manna MD PhD2*, Ilaria Contaldo MD1, Domenico M. Romeo MD1, Maria Chiara Stefanini MD1, Antonio Chiaretti MD3, Eugenio Mercuri MD PhD1 and Paolo Mariotti MD1

Departments of 1Pediatric Neurology, 2Internal Medicine, and 3Pediatrics, Catholic University, Gemelli Hospital, Rome, Italy
4Child and Adolescent Neuropsychiatry Unit and Laboratory of Molecular Psychiatry and Neurogenetics, University Campus Bio-Medico, Rome, Italy

KEY WORDS: intravenous immunoglobulin therapy (IVIG), neuropsychiatric systemic lupus erythematosus (NPSLE), Epstein-Barr virus (EBV), pediatric psychosis

PATIENT DESCRIPTION

A previously healthy 7 year old boy was admitted to our Pediatric Neurology Unit due to abrupt and persistent dystonic movements in the left leg associated with paresthesia and difficulty walking. His family history was positive for epilepsy (maternal grandfather) and immunological disease (his mother was affected by Hashimoto thyroiditis and autoimmune gastric atrophy). He was born at term after an uncomplicated pregnancy. His peripheral course and neurodevelopmental milestones were reported as normal. The child had an uneventful medical history except for sporadic headaches and oral ulcers. Seven days before emergence of the motor disorder he experienced some prodromal symptoms, including mild fever, pharyngitis, headache, cough and fatigue, which were treated with antibiotics. In the following days the child exhibited dystonic and uncoordinated distal jerking movements in the left arm, photophobia, diplopia and clumsiness. He was also noted to have some psychiatric symptoms: irritability, mood disturbance, psychomotor agitation and hallucinations. Anti-epileptic therapy was begun (intravenous levetiracetam followed by oral levetiracetam and clobazam), and although there was a mild improvement the psychiatric symptoms failed to respond to antipsychotic therapy (risperidone). In the psychiatric symptoms failed to respond to antipsychotic therapy (risperidone). In
the following days his clinical status was critical due to the appearance of frontal lobe signs, severe cognitive impairment, severe axial hypotonia, facial grimacing, and difficulty eating and performing activities of daily living.

Brain and medulla MRI showed persistent cortical and subcortical leptomeningeal enhancement, whereas SPECT and PET-CT (positron emission computed tomography) total body imaging excluded a paraneoplastic syndrome and alterations in both cerebral perfusion and metabolism. Bone marrow biopsy was negative for lymphoproliferative disorder or B lymphocyte clonality. Electromyography, sensory/motor nerve conduction and auditory evoked potentials were normal. Only the motor evoked potential study showed cortical dysfunction. He presented also with malar rash, arthralgia and oral ulcers. When immunological assessment was repeated, ANA, anti-dsDNA and antinucleosome antibodies were positive (ANA at 1/80 dilution with speckled pattern, anti-dsDNA Ab 22.4 AU/ml, anti-nucleosome Ab120 UA/ml, cANCA 4.7 UA/ml). Other laboratory tests, including complement C3-C4, renal function (creatinine levels), antiphospholipid antibodies and white blood cell count (WBC), were negative except for erythrocyte sedimentation rate (ESR). According to criteria of the American College of Rheumatology (ACR, 1997) and the International Collaborating Clinics Classification (SLICC, 2012), NPLES diagnosis was fulfilled due to the presence of three clinical criteria (malar rash, oral ulcers, neurological involvement) and two immunological criteria (ANA, anti-dsDNA positivity). The presence of antinucleosome antibody positivity, known to be a sensitive and highly specific marker for SLE (mainly lupus nephritis), further confirmed this diagnosis.

In view of clinical worsening and immune mediated etiopathogenesis, intravenous immunoglobulin therapy (IVIG) was

---

**Table 1. Laboratory assessment before IVIG treatment**

<table>
<thead>
<tr>
<th>Serums tests</th>
<th>At onset</th>
<th>One month later</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR, C-reactive protein, serum lactate, hepatic enzymes, ammonium, ASO titer, rheumatoid factor, lactate, creatinine, WBC Serum ceruloplasmin, copper serum and urine levels Toxoplasma, HSV 1/2, CMV, VZV, HHV6, B. borrelia, Mycoplasma IgM/MgG, larva migrans, VDRL EBV serology</td>
<td>All negative</td>
<td>All negative, except for ESR (32 mm)</td>
</tr>
<tr>
<td>VCA IgG 68.80 UA/ml, VCA IgM+, EBNA IgG &gt; 200 UA/ml</td>
<td>All negative</td>
<td>All negative</td>
</tr>
</tbody>
</table>

**CSF analysis**

<table>
<thead>
<tr>
<th>Macroscopy</th>
<th>Milder opalescent CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>Negative</td>
</tr>
<tr>
<td>Glucose</td>
<td>Negative</td>
</tr>
<tr>
<td>Protein</td>
<td>Negative</td>
</tr>
<tr>
<td>Lactate</td>
<td>Negative</td>
</tr>
<tr>
<td>WBC</td>
<td>Negative</td>
</tr>
<tr>
<td>Oligoclonal cells</td>
<td>Negative</td>
</tr>
<tr>
<td>Cancer cells</td>
<td>Negative</td>
</tr>
<tr>
<td>Blood-brain barrier</td>
<td>Negative</td>
</tr>
<tr>
<td>PCR (HSV 1/2, CMV, Enterovirus Parvovirus, Enterovirus, HSV 1/2, CMV, VZV, HHV6, ICV virus, B. borrelia) PCR EBV</td>
<td>DNA+</td>
</tr>
</tbody>
</table>

**Immunological assessment**

<table>
<thead>
<tr>
<th>CD4/CD8 ratio</th>
<th>2.25 (1.10–1.80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TPO Ab</td>
<td>Negative</td>
</tr>
<tr>
<td>ABMA, AMAs, anti-centromere, anti-SCL 70, anti-citrulline, anti-endomyosial Ab, anti-Jo 1 Ab, anti-transglutaminase Ab, cryoglobulins, immune complexes, C3, C4, anti-pANCA, ENA, ANA, anti-dsDNA</td>
<td>All negative except for:</td>
</tr>
<tr>
<td>Anti-AGNA, anti-Me antibodies</td>
<td>All negative</td>
</tr>
<tr>
<td>Anti-Yo, anti-Ri, anti-Hu, anti-CCU2, anti-AGNA, anti-Me antibodies</td>
<td>Unavailable</td>
</tr>
<tr>
<td>Anti-NMDAR, anti-GABA b, anti-Lgi-1, anti-CASPR 2 antibodies</td>
<td>Unavailable</td>
</tr>
</tbody>
</table>

**Antiphospholipid antibodies**

<table>
<thead>
<tr>
<th>Lupus anticoagulant, anti-β2 glycoprotein, anti-cardiolipin, anti-prothrombin antibodies</th>
<th>Negative</th>
</tr>
</thead>
</table>

**Onconeural antibodies**

<table>
<thead>
<tr>
<th>Anti-Yo, anti-Ri, anti-Hu, anti-CCU2, anti-AGNA, anti-Me antibodies</th>
<th>Negative</th>
</tr>
</thead>
</table>

**Antineuron antibodies**

<table>
<thead>
<tr>
<th>Anti-NMDAR, anti-GABA b, anti-Lgi-1, anti-CASPR 2 antibodies</th>
<th>Negative</th>
</tr>
</thead>
</table>

**Bold indicates EBV infection and Lupus positivity**

Ab = antibodies, ERS = erythrocyte sedimentation rate, HSV = herpes simplex virus, CMV = cytomegalovirus, WBC = white blood cell count, ASMA = anti-smooth muscle antibodies, AMA = anti-mitochondrial antibodies, ENA = anti-extractable nuclear antigens antibodies, pANCA-C = anti-neutrophil cytoplasmic antibodies, dsDNA = double-stranded DNA, anti-TPO = anti-thyroid peroxidase antibodies, anti-T = anti-thyroglobulin antibodies, NMDA = N-methyl-D-aspartate, GABAB = γ-aminobutyric acid receptor B, LGI1 = glioma inactivated protein-1, CASPR2 = contactin-associated protein-2, VCA = viral capsid antigen, TB = tuberculosis, VZV = varicella zoster virus, B. borrelia = Borrelia burgdorferi
started (0.4 g/kg/day) for 5 days, repeated 20
days later for a total of three times. Steroids
were discontinued following therapy with IVIG. During the course of the three
IVIG administrations, the child showed a
progressive improvement in both clinical
and immunological tests with progressive
reduction of lupus antibody titers in
subsequent months. He started to speak
fluently, regained walking ability, improved
in activities of daily living and in behavior
with a reduction of psychotic symptoms.

One year later, there was no evidence of
hemiparesis and cognitive assessment was
in the borderline range, with difficulties
mainly in working memory and visuoper-
ceptual ability. The child continued to have
episodic malar rash without the presence
of renal or respiratory symptoms or levels
of active disease required for corticosteroid
therapy. Laboratory tests revealed only
ANA positivity at a dilution of 1/40 (non-
specific speckled pattern) with the other
LES autoantibodies in the normal range.

**COMMENT**

Autoimmune diseases are believed to result from interactions between genetic
and environmental factors. Among them are ultraviolet light, drugs, vaccinations,
smoking, and infectious pathogens, which could directly affect the immune system
or induce epigenetic changes modulating gene expression. Infections could act as envi-
ronmental triggers inducing or promoting SLE in genetically predisposed individuals.
EBV is one of the environmental risk fac-
tors most closely associated with SLE [1].
Several case-control studies provide more
evidence of EBV infection as a triggering
pathogenic factor in adult and juvenile SLE
patients, i.e., abnormally elevated EBV load
in their blood and higher EBV antibody
titer as compared with healthy controls
[1,2]. Conversely, other cohort studies did
not find evidence of a correlation between
primary infection of EBV and SLE, sug-
gestting an immune dysregulation under-
lying the immune status of SLE patients.
However, the role of latent and EBV reacti-
vation in the autoimmune response of SLE
was recently investigated and our clinical
case seems to confirm this association.

As with other types of herpes, periodic
EBV reactivation can occur, stimulating the
immune system and leading to autoimmune
disease in genetically predisposed indi-
viduals. After primary infection and the
lytic phase, autoreactive B cells proliferate,
leading to latent infection. EBV-infected
memory B cells are resistant to apoptosis
due to the expression of virus-encoded
anti-apoptotic molecules. These cells act
as antigen-presenting cells stimulating T
cell migration and production of cytokines
such as IL-10, which promote EBV-infected
B cell proliferation and inhibit cytotoxic
T cell lysis of EBV-infected cells [1,2].
Specifically, it was found that patients with
SLE, compared with healthy controls, had
an increased frequency of EBV specific
memory CD4+ T cells that produce interferon
as a compensatory mechanism for an
inadequate CD8+ T cell response against
EBV, suggesting defective control of latent
EBV infection in SLE [2]. These mechanisms
could lead to subsequent infection and
transformation events resulting in chronic
damage and altered autoimmune responses.
EBV antigens can lead to autoimmunity by
structural molecular mimicry with common
SLE antigens. It is known that antibodies
against EBNA1 could cross-react with the
spliceosomes SmD and 60 Kd Ro [1,2], and
in a murine study anti-EBNA antibodies
produced after immunization cross-react
with dsDNA leading to development of
lupus antibodies.

EBV could also act through “functional”
molecular mimicry, with critical immune
regulatory components interfering with
immune surveillance and latent process
through two latent membrane proteins
(LMP-1, 2) and by transactivation of
HERV-K18 superantigen activity [2].
Anti-EBNA-1 antibodies are expressed in
the latent phase of EBV infection and are
responsible for tethering the EBV genome
to chromosome-altering gene expression.
In our patient, the presence of anti-EBNA
antibodies was suggestive of a previous
infection, and the presence of EBV DNA in
the CSF confirmed EBV encephalitis that
preceded the neuropsychiatric symptoms
and the autoantibodies typical of SLE
(anti-dsDNA, ANA, and antinucleosome
antibodies).

NPSLE is a common SLE manifestation
occurring mainly in children at SLE onset
with different symptoms: psychosis, affect-
tive disorders, seizures, cerebrovascular
disease, movement disorder, acute confused
state, organic brain syndrome (disturbance
of memory, perception, orientation, or
other cognitive function) and neuromyelites
[1]. These clinical sequelae are thought to
arise from vascular abnormalities or an
interaction of neuronal autoantibodies
with neuronal and glial cells that lead to
diffuse neuropsychiatric manifestations,
such as psychosis and acute confused state
because of the development of inflamma-
tory mediators. NPSLE has been reported
to occur more frequently in pediatric SLE
patients (22–95%) and is correlated with
higher mortality and poor outcome due to
its resistance to treatment [1].

Our patient had a severe neuropsychiat-
ric condition resistant to the common
psychotropic medications and to the
standard therapy with corticosteroids.
However, the child responded very well
to IVIG treatment, with resolution of the
neurological picture. IVIG has been used
in pediatric patients with a severe type
of autoimmune diseases (SLE included),
encephalopathy and immune related
motor disorder [3]. In our patient IVIG
treatment led to prompt resolution of the
neurological clinical signs together with a
reduction in antibody pattern of SLE. IVIG
is known to also play a neuroprotective role
by interfering with the anti-idiotypic net-
work [3-5] and can act through different
mechanisms: autoreactive B lymphocyte
suppression, neutralization of B cell acti-
vation cytokines (reduction of IL-10 and
IL-6 secretion), hypoaclivation of TLR-9
in B cells, and modulation of the immune
response [4,5]. Interestingly, in our patient,
we found high levels of antinucleosome
antibodies, known to be a better diagnos-
tic marker than anti-dsDNA antibodies for
SLE, especially in children. In addition, we
found higher levels of neutrotrophins NGF.
(nerve growth factor) and BDNF (brain-derived neurotrophic factor) in the CSF of our patient, observed to be deregulated in the serum of SLE patients compared to healthy controls and associated with psychotic symptoms in NPLES.

In conclusion, EBV reactivation can trigger a clinical and laboratory picture of NPLES probably by inflammatory mediators. Therefore, SLE immunological assessment, including antinucleosome antibodies, should be routinely checked in patients with neuropsychiatric signs of EBV reactivation. IVIG should be considered, mainly in children, when standard therapy fails.

Correspondence
Dr. C. Brogna
Dept. of Pediatric Neurology, Catholic University,
L.go Agostino Gemelli n 1, Gemelli Hospital,
Rome, Italy
Phone: +390630156307
Fax: +390630154363
email: claudiabrogna@yahoo.it;
c.brogna@unicampus.it

Acknowledgment
We thank Dr. F. Scuderi for her contribution in the laboratory assessment.

References

In the case of autoimmune diseases, such as type 1 diabetes, so-called exhausted T cells may be the answer to stopping disease. Long et al. report that the best responses in type 1 diabetics treated with teplizumab, a monoclonal antibody against CD8, were associated with CD8+ T cells with features of exhausted T cells. These cells recognized a broad spectrum of autoantigens but proliferated less than non-exhausted cells ex vivo. However, they were not terminally exhausted: stimulation with a ligand for the inhibitory receptor TIGIT further down-regulated their activation. Inducing T cell exhaustion may thus represent a potential therapeutic approach in type 1 diabetes.

*Sci Immunol* 2016; 1: eaai7793

Capsule

**Status alters immune function in macaques**

Rhesus macaques experience variable levels of stress on the basis of their position in the social hierarchy. To examine how stress affects immune function, Snyder-Mackler et al. manipulated the social status of individual macaques. Social status influenced the immune system at multiple levels, from immune cell numbers to gene expression, and altered signaling pathways in a model of response to infection. Macaques possess a plastic and adaptive immune response wherein social subordination promotes antibacterial responses, whereas high social status promotes antiviral responses.

*Science* 2016; 354: 1041

Eitan Israeli

Capsule

**Exhausting autoimmunity in type 1 diabetes**

In the case of autoimmune diseases, such as type 1 diabetes, so-called exhausted T cells may be the answer to stopping disease. Long et al. report that the best responses in type 1 diabetics treated with teplizumab, a monoclonal antibody against CD8, were associated with CD8+ T cells with features of exhausted T cells. These cells recognized a broad spectrum of autoantigens but proliferated less than non-exhausted cells ex vivo. However, they were not terminally exhausted: stimulation with a ligand for the inhibitory receptor TIGIT further down-regulated their activation. Inducing T cell exhaustion may thus represent a potential therapeutic approach in type 1 diabetes.

*Sci Immunol* 2016; 1: eaai7793

Eitan Israeli

Capsule

**Cardiac side effect**

Antibodies that block CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) and PD-1 (programmed death 1) allow T cells to launch antitumor immune responses. Although these checkpoint inhibitors improve survival in melanoma patients, inflammation of other tissues is a common side effect. Johnson et al. report that two melanoma patients treated with a combination of the checkpoint inhibitors developed fatal cardiac damage. Biopsies revealed that T cells and macrophages that infiltrated the heart were the same as those found in skeletal muscle and the tumor. Neither patient had cardiac risk factors other than hypertension. Review of a safety database suggests that severe myocarditis from such combination therapy affects less than 1% of patients. The mechanism for this rare toxic effect is not known.


Proc Natl Acad Sci USA 2016; 10.1073/pnas.1603325113

Eitan Israeli