The immune system in pediatric seizures and epilepsies: current state of knowledge

KORFF, Christian

Abstract
This thesis aims at presenting an overview of the current state of knowledge on the involvement of immunity in pediatric epilepsies. In particular, we emphasize the role, suspected or confirmed, of various auto-AB discovered in children with seizures. For that purpose, we first place our review in a brief historical perspective, and describe certain illustrative inflammatory and autoimmune systemic and neurological diseases in which seizures are a hallmark. The current knowledge of the pathophysiological mechanisms of the link between inflammation and seizures is summarized. We then discuss some of the childhood epilepsies and encephalitides with seizures in which specific auto-AB are involved. Finally, therapeutic options and future perspectives are presented.

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"The immune system in pediatric seizures and epilepsies: current state of knowledge"

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Christian M. KORFF

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To Simona, Clelia, Alexandra, and Maximilien
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The immune system in pediatric seizures and epilepsies: current state of knowledge

Christian M. Korff

Pediatric Neurology, University Hospitals, Geneva

Correspondence: Dr C. Korff, Pediatric Neurology, University Hospitals Geneva, 6 Rue Willy-Donzé, CH 1211 Geneva 14, Switzerland
T: +41 22 372 45 72, F: +41 22 372 54 89, E: christian.korff@hcuge.ch

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1. Introduction

The immune system has been long involved in systemic diseases in which neurological symptoms, such as seizures, may be observed. These include systemic lupus erythematosus (SLE), celiac disease, Hashimoto thyroiditis, and type-1 diabetes, among many more. In a recent large population-based study (N=2’518’034), children with autoimmune diseases had an overall 5-fold increased risk of epilepsy compared with age-matched controls.1 The risk of epilepsy was consistently heightened in all of the 12 different autoimmune diseases that were considered, including some not known to affect central nervous system function, such as myasthenia gravis or psoriasis.1 In some of these situations, specific autoantibodies (auto-AB) have been involved in the development of neurologic signs and symptoms, yet precise pathophysiological mechanisms remain to be identified. In addition, immune function has been intensively studied in numerous primary neurological diseases, which include the frequent epilepsies for which an underlying etiology remains to be discovered.2 This productive research activity rapidly led to an ever increasing level of understanding, and to the universal acknowledgement of the importance of immunity in the pathophysiology of the epilepsies, as illustrated by the current intention of the International League Against Epilepsy (ILAE) to include a new immune etiological category in its proposal for an Organization of the Epilepsies.3 Overall, these discoveries open encouraging management and therapeutic perspectives for an important number of patients with seizures. As often, however, most studies concern adults, and data in children remain scarce.4

This article aims at presenting an overview of the current state of knowledge on the involvement of immunity in pediatric epilepsies. In particular, we emphasize the role, suspected or confirmed, of various auto-AB discovered in children with seizures. For
that purpose, we first place our review in a brief historical perspective, and describe certain illustrative inflammatory and autoimmune systemic and neurological diseases in which seizures are a hallmark. The current knowledge of the pathophysiological mechanisms of the link between inflammation and seizures is summarized. We then discuss some of the childhood epilepsies and encephalitides with seizures in which specific auto-AB are involved. Finally, therapeutic options and future perspectives are presented.

2. Historical perspective

According to Jankovic, the earliest experiences establishing a potential link between epilepsy and inflammation were those performed by Delezenne at the beginning of the 20th century.\textsuperscript{5,6} This former director of the physiology laboratory at the Institut Pasteur in Paris induced clonic and tonic convulsions in dogs by injecting in their frontal lobes duck antisera against various parts of the central nervous system, whereas no clinical manifestations were observed in dogs to which normal serum was applied.\textsuperscript{5-7} The development of numerous tools and technical skills in the decades that followed allowed to progressively confirm and further precise these initial discoveries. In the 1960’s, intraventricular injections of various anti-brain AB to different animals produced local or multifocal changes of electrographic activity, which included long-lasting epileptiform discharges in the hippocampus of those who had received anti-hippocampus AB.\textsuperscript{5,8-10} In another study performed in the same decade, immunofluorescent microscopy analysis of guinea pig cortex slices, to which serum samples of epileptic and normal rabbits were applied, showed that positive binding to grey matter was significantly more frequent with serum samples of epileptic animals than with sera of normal animals.\textsuperscript{11} The results of similar experiments performed at the beginning of the 1970’s were summarized in a
hypothesis article published in 1976. In that publication, additional elements in favor of an immune component in epilepsy included lymphocytic infiltration at the site of application of aluminium hydroxide, one of the widely used agent in experimental epilepsy, also known as an adjuvant for antibody production; eosinophilia reported in certain children with epilepsy; and decreased serum levels of IgA in children with febrile seizures. Further steps towards current understanding were taken with the study of children with febrile seizures, the frequent observation of seizure exacerbation during febrile and infectious diseases in patients with epilepsy, as well as discovery of anticonvulsive properties of certain anti-inflammatory drugs, like ACTH and steroids that induced a favorable response in certain severe epilepsies of childhood, such as West syndrome. In addition, numerous descriptions of systemic autoimmune diseases in which seizures often represent one of the major signs of CNS involvement, such as systemic lupus erythematosus (SLE) and celiac disease (CD), were reported. Finally, the study of certain “pure” neurological chronic and progressive inflammatory disorders, such as Rasmussen encephalitis (RE), greatly contributed to better understand the immune bases of certain childhood epilepsies. The interplay between immunity and seizures in SLE, CD, and RE are summarized hereafter, with specific emphasis given on data in children.

3. Systemic autoimmune diseases with seizures

3a. Systemic lupus erythematosus

SLE is an autoimmune systemic disease, in which CNS manifestations occur in up to 75% of adult patients, and in 22 to 95% of children. Its etiology is unknown, and although the accepted final step of the pathway of CNS symptoms is microvasculature involvement, detailed pathophysiological mechanisms underlying cerebral dysfunction remain to be discovered. A recent study on TREX1
analysis (a gene responsible of the synthesis of an exonuclease involved in cell death processes, DNA degradation, and response to oxidative stress) in 8370 SLE patients and 7340 controls, showed that certain single nucleotide polymorphisms (SNPs) were significantly associated with neurological manifestations, and especially seizures, in SLE patients.\textsuperscript{18} Interestingly, the same risk haplotype was associated with the presence of certain auto-AB in some of the ethnic subpopulations studied.\textsuperscript{18} Seizures occur in 4-51\% of SLE patients in general,\textsuperscript{1,19-21} and may be more frequent in children than in adults.\textsuperscript{1} In the recent population-based study by Ong and coll., 7.3\% of the 123 children with SLE had epilepsy, a risk 21.6 times higher than in the general population. Adults also had an increased risk, but to a lesser extent: 2.5\% of the 9696 patients with SLE had epilepsy, a risk 7.4 higher than in controls.\textsuperscript{1} In a prospective study on 75 pediatric-onset SLE patients, epilepsy was diagnosed in 15\%, and isolated seizures had occurred in 47\% of the entire cohort, after a 6-year follow-up.\textsuperscript{16} In a previous cross-sectional study on a smallest cohort of 24 Oriental children with SLE, 18 of them (75\%) had neurological manifestations, among which 11 (61\% of those with neurological manifestations, and 46\% of the total number) had seizures.\textsuperscript{22} Among all AB studied in SLE, some of them seem to be particularly associated with seizures. This is the case of anticardiolipin (aCL) AB, which titers were more frequently highly positive (i.e. > 5 standard deviations) in patients with epilepsy than in those without seizures, in a group of 252 teenagers and adults with SLE.\textsuperscript{23} In that study, all of the epileptic patients were also positive for anti-nuclear AB, 76.2\% had anti-DNA AB, and the risk of having seizures with highly positive aCL AB was 3.7 times more important than if aCL AB levels were not detectable. A significant association of high titers of aCL AB and seizures was also found in 163 unselected epileptic teenagers and adults without SLE followed at the Centre Saint-Paul in
Based on previous animal studies, Liou et al. hypothesized that aCL AB exerted their proconvulsant effect either through an inhibition of the GABA-receptor ion-channel system, or via a specific cross-reaction with certain brain phospholipids.

### 3b. Celiac disease

Celiac disease (CD), or gluten intolerance, is an immune-mediated disease of the small bowel, characterized by lymphocyte infiltration of the intestinal mucosa, crypt-cell hyperplasia, and villous atrophy. It is one of the most frequent chronic diseases in childhood. Clinical manifestations appear after gluten ingestion in genetically susceptible individuals. Neurological and psychiatric symptoms, such as peripheral neuropathy, ataxia, dementia, and epilepsy, have been associated in up to 22% of patients with CD. More specifically, in the study by Ong and coll., epilepsy was present in 5.7% of 261 children with CD. Cerebral imaging may reveal calcifications, or isolated white matter lesions, often (but not exclusively) located in the posterior regions of the brain, even in patients without overt intestinal symptoms. Preliminary studies focused on the role of malabsorption-induced vitamin or folate deficiencies in the appearance of extraintestinal symptoms of CD. Because neurological symptoms can arise without enteropathy, autoimmune processes may better explain their appearance, however. A central nervous vasculitis characterized by pial angiomatosis, fibroded veins and vascular microcalcifications has been described in certain CD patients, for example. In addition, circulating auto-AB considered as markers for CD, such as anti-transglutaminase (TG), anti-gliadin, anti-reticulin, and anti-endomysium AB, were found at various frequencies in CD patients with extraintestinal symptoms. Anti-gliadin IgA or IgG and anti-reticulin IgA, for instance, were found in 17 (8.3%) and 1 (0.5%), respectively, of 206 children with
various unspecified neurological disorders. In that subpopulation, the CD prevalence rate was calculated at 14.6/1000, a much higher frequency than that of the general population. Anti-TG6 AB may be more specific for neurological involvement, and have been particularly well described in relation with gluten ataxia. More specifically, several studies addressed the relation between seizures and epilepsies and CD in children. In one of them, anti-gliadin or anti-endomysium AB were found in 2 (8%) of 25 children with focal epilepsies and occipital spikes, but in none of the patients with focal epilepsies and central-temporal spikes. Of particular interest, auto-AB against TG6 were recently found in a patient with CD, epilepsy and cerebral calcifications (CEC syndrome). The authors of that study demonstrated antibody binding to cerebral and cerebellar neuronal and glial tissue by indirect immunofluorescence, thereby adding to the suspicion of a specific role of TG6 autoimmunity in the pathophysiology of neurological manifestations in CD. In a cohort study, though, the prevalence of anti-TG and anti-gliadin AB was identical in a group of 272 children with epilepsy as compared to 300 age-matched healthy children, a similar finding to what had been previously reported in a large controlled group of adults with epilepsy. Thus, the precise role of auto-AB in the pathophysiology of seizures in CD remains to be explained.

4. Neurological autoimmune diseases with seizures: the model of Rasmussen encephalitis

Rasmussen encephalitis is an immune-mediated disorder of the central nervous system, characterized by refractory focal epilepsy, progressive hemispheric atrophy and contralateral neurological dysfunction. Advances in the level of pathophysiological understanding led to the publication of consensus diagnostic criteria and management guidelines in 2005. The initial results of basic research
studies on RE focused on the potential role of humoral immunity and, in particular, of auto-AB against the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subunit of glutamate receptor 3 (GluR3). This primary hypothesis was reinforced by additional findings, such as the presence of oligoclonal bands in the CSF of some RE patients, and the good response to immune therapies such as plasma exchanges. Subsequent studies challenged these findings, however, first because AB against GluR3 were not present in all RE patients, and second because they were also found in other types of epilepsies. In two reports, the presence of IgG or IgM AB against the ε2 subunit of NMDA-GluR (GluRε2) was demonstrated in the serum or the CSF in 19 of 20 patients with RE. Here again, these AB were considered as non-specific markers for the disease, rather than being directly responsible for its underlying pathological processes. The presence of peripheral T-cells stimulated by GluRε2, demonstrated in some patients, confirmed that cellular immune response was involved in RE, as previously suspected on the basis of histological findings showing the predominance of T-cell infiltration of the CNS in these patients. More precisely, a Granzyme-B mediated T-cell cytotoxic reaction was shown to play a role in the progressive tissue loss of RE patients. This mechanism per se cannot explain the genesis of seizures, however. Current hypotheses and knowledge about the immunopathological bases of RE were recently summarized in a review paper by Varadkar and coll. These mechanisms include antibody-mediated parenchymal degeneration (that remains to be formally demonstrated and clarified); T-cell (mainly CD8) cytotoxicity, driven by an antigen which exact nature remains to be discovered; activation of astrocytes and microglial cells; and increased expression of certain genes involved in T-cell activation, and their recruitment on the site of inflammation by specific chemokines.
Thus, the immune mechanisms underlying RE are likely to be mixed, and the genesis of seizures in that condition remains to be clarified.40,50,51

5. Immunity and epilepsies: recent progress in the understanding of a complex relationship

As a result of the above-mentioned initial observations, an explosion of the number of scientific studies on the relation of autoimmunity and epilepsy has occurred since the early 1990’s (Figure 1). In some circumstances, immune activation precedes and provokes the appearance of seizures. In other situations, contrarily, the inflammatory cascade is activated by seizures themselves. It is generally accepted that a certain degree of immune reaction is favorable, and contributes to protect the brain from permanent damage after seizures; in certain circumstances, however, these immune processes may be deleterious.52 The purpose of the next paragraphs is to discuss these complex aspects.

5a. Are seizures a consequence or a cause of immune activation?

It is now generally accepted that inflammation can be both the consequence and the cause of seizures, and that the activation of the immune system can induce permanent functional changes in the CNS, which may themselves contribute to generate epilepsy.53,54 A detailed description of the molecular mechanisms involved in the immune cascade related to seizures and epilepsy is beyond the scope of this review. On the basis of four recent and beautifully illustrated overview articles,53,55-57 one can nevertheless attempt to summarize its most important steps, as follows (Figure 2):

1. An initial injury occurs in the CNS or in the periphery, and provokes the activation of an inflammatory response. Various events have been identified as being able to play such a role, which include peripheral infections or autoimmune diseases, CNS
vascular disease (thromobosis, emboli, hemorrhage), vasculitis, metabolic disorders, infections, seizures and status epilepticus.

2. Inflammatory mediators are released in either compartment, or in both, depending on the nature of the initial injury. These mediators include various cytokines (such as interleukin (IL)-1β, IL-6, and tumor-necrosis factor (TNF)-α), complement proteins, so-called “danger signals” (molecules that “alert” the microenvironment about an ongoing injury, such as high-mobility-group box (HMGB)-1 and its activation of Toll-like receptor 4 (TLR4) in neurons and glial cells), cell adhesion molecules, proteins of the cyclooxygenase (COX)-2 signaling pathway, and chemokines. Their upregulation and release by lymphocytes in the periphery, or by activated glial and neuronal cells, may in turn provoke blood-brain-barrier (BBB) breakdown, adhesion and penetration of activated peripheral lymphocytes, immunoglobulins and albumin into the brain (and, for the latter, subsequent activation of the transforming growth factor (TGF)-β signaling pathway), increasing extracellular potassium concentration, as well as functional changes in neurons, glial cells, and astrocytes.

3. Examples of these functional changes include the increased expression of IL-1R1 (the target and mediator of the biological response to IL-1β) in neurons; the activation of various intracellular kinase families, such as that inducing phosphorylation of the NR2B subunit of glutamatergic N-methyl-D-aspartate (NMDA)-receptors (NMDA-R); the inhibition of the glutamate reuptake, or the increase of its release in the extracellular space by astrocytes; the promotion of synaptic reorganization; and the dysfunction of ion channels. Animal research studies also showed that certain genes that code for mediators of the inflammatory response, such as IL-1, IL-6 (and its receptor) or IL-1β are upregulated in the acute phase that follows status epilepticus or traumatic brain injury.52,58
All of these mechanisms induce an increase in neuronal excitability, a lower seizure threshold, and thereby may engender a vicious cycle resulting in a chronic state of seizure susceptibility. The various mechanisms for inflammation-induced epileptogenesis have been further detailed following interesting animal research findings. These include the above-mentioned increased adhesion of activated peripheral leukocytes to endothelial cells, followed by their infiltration into the CNS through cytoskeletal reorganization. These cells generate free radicals and cytotoxic enzymes, which, in addition to further production and secretion of cytokines and chemokines, participate to neuronal dysfunction or degeneration that contribute to the appearance of a subsequent chronic susceptibility to seizures.

From another standpoint, the relation between the BBB and the occurrence of epilepsy has been studied extensively for years, but the way by which BBB disruption may provoke chronic changes at the basis of epilepsy has remained incompletely understood. It has been hypothesized, for example, that acute BBB disruption after initial seizures cause prolonged or permanent brain permeability changes at the basis of chronic surrounding neuronal excitability and further seizure genesis. Recent advances in the “BBB disruption theory” are likely to help our understanding of the process. Indeed, Bargerstock and coll. showed that S100B, an astrocytic protein released in the systemic circulation in case of disruption of the BBB endothelial tight-junctions, such as that observed during seizures, may in fact induce a systemic autoimmune reaction against the brain, at the basis of chronic CNS diseases such as epilepsy and Alzheimer disease. These exciting results need confirmation.

Finally, various experiments studying epigenetic modifications related to the acute phase after provoked seizures are being conducted. Whether such modifications play
a role in the development of epilepsy after an initial insult remains to be demonstrated.\textsuperscript{58}

5b. Are there clinical situations in which seizures beget seizures and epilepsy through inflammatory mechanisms?

The clinical example of febrile seizures and their occasional association with subsequent epilepsy may shed some light on that question that remains open.

**Febrile seizures and mesial temporal sclerosis**

Febrile seizures (FS) are the most frequent type of seizures in childhood. They concern 2-5\% of children in the United States and Western Europe,\textsuperscript{69} and it is esteemed that up to 15\% of them are prolonged or progress into febrile status epilepticus (FSE, defined as a seizure lasting for more than 30 minutes without interruption or repeated seizures without recovery of previous state between events).\textsuperscript{70} Some of the children with prolonged FS or FSE later develop mesial temporal (or hippocampal) sclerosis and related refractory epilepsy. According to recent data, those at particular risk are children whose acute MRI (performed in the 72 hours that follow the SE episode) shows hippocampal T2 hypersignals in the temporal lobe, a finding significantly associated with focal slowing or attenuation in the same region on acute EEG.\textsuperscript{70-72} Similarly, up to 20\% of children with epilepsy originating in the temporal lobe had previous FS.\textsuperscript{69} The relationship between febrile seizures and hippocampal lesions is complex, and the question as to which precedes the other is unresolved. Experimental and clinical data suggest that some of the inflammatory mechanisms described above may underlie this evolution.\textsuperscript{70} In particular, IL-1\(\beta\), shown to be released during febrile illnesses and after FS,\textsuperscript{73} was found in higher concentrations in the hippocampus of rats that had developed epilepsy after FSE than in those that had not.\textsuperscript{74} This observation may be in favor of a
seizure-induced epileptogenic inflammatory cascade, which remains to be formally
demonstrated and clarified, though. Additional cytokines, such as TNFα, IL-6, IL-1β or
IL-10, and have been found in the blood or CSF of patients after FS, but here
again, it is unclear whether their presence is a cause or consequence of seizures,
and their potential role in subsequent development of epilepsy has not been formally
demonstrated. Finally, 19 inflammation-related genes were recently tested in 98
patients with febrile seizures and compared to those of 123 patients without seizures.
Significant associations with febrile seizures were reported for genes encoding the
purinergic receptor P2X7, TLR4, the IL6-receptor, and the subtype EP3 of the
prostaglandin E receptor 3.

5c. The role of auto-AB in the inflammatory cascade

The presence of auto-AB in the serum or CSF of patients with seizures and, more
specifically, their precise role and position in the inflammatory cascade described
above, remain to be fully understood. None of them seems associated with any
specific imaging or EEG feature, and inflammatory parameters are often lacking in all
compartments (Table 1). It is generally accepted that some auto-AB may directly
contribute to the genesis of seizures, for example by inducing dysfunction of ion
channels or CNS receptors and consequent changes in local ionic and
neurotransmitter homeostasis, whereas others “simply” represent markers of an
immune disorder. It seems from the rare immunopathological studies performed to
date, that the type of immune response depends on the target of the auto-AB
involved. In one recent report, Bien and coll. showed that AB directed against certain
intracellular antigens, such as the pre-synaptic enzyme glutamic acid decarboxylase
(GAD)-65, induced a Granzyme B T-cell mediated cytotoxic reaction (very similar to
that observed in patients with Rasmussen encephalitis); contrarily, certain AB
directed at surface antigens, such as proteins of the voltage-gated potassium channel (VgKC), rather induced a B-cell based AB- and complement-mediated destruction of neurons. Interestingly, other auto-AB of the same group, such as those directed against the NMDA-R, neither showed signs of involvement of T-cells, nor of the complement system.\textsuperscript{77} According to previous studies, these latter AB may play a pathogenic role by reducing the expression of NMDA-R, in particular in the hippocampus.\textsuperscript{78} As mentioned by Bien and coll., however, this mechanism is unlikely to explain all symptoms of the complex clinical picture observed in these patients (see below). Whether a more diffuse or multifocal inflammatory reaction is present at another point of their evolution remains to be demonstrated.\textsuperscript{77}

5d. Auto-AB related to seizures in childhood (Based on \textsuperscript{79}, with authorization)

5d.1) Intracellular targets

Glutamic acid decarboxylase (GAD)

Certain patients with neurological symptoms produce auto-AB directed against GAD (GADA), a pre-synaptic intracellular enzyme involved in the synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GADA may be found in their serum, their CSF, or both. Clinically, these patients may present with various conditions, which include type-1 diabetes, stiff-person syndrome, ataxia, or signs of acute immune encephalitis, such as cognitive troubles and seizures.\textsuperscript{80,81} The demonstration of an intrathecal AB production, as well as of higher levels of GADA, seems to correlate with the presence of neurological symptoms.

As mentioned above, Bien and coll. demonstrated T-cell neurotoxicity in patients with GADA-encephalitides.\textsuperscript{77} The exact mechanisms by which GADA may produce seizures remain unclear, though. A decreased concentration of cortical GABA, which suggests related increased excitability, was demonstrated by MRI spectroscopic
analysis in certain patients. In addition, the injection of GADA-positive serum or CSF of patients in healthy animals reproduced similar symptoms in the latter. Given the intracellular activity of the enzyme they target, and the high frequency of GADA in diabetic patients without overt neurological manifestations, the direct pathogenic role of GADA has been challenged, however. In fact, it is generally considered that GADA rather represent biomarkers of an autoimmune reaction of unknown origin. Imaging and EEG findings are non-specific. Predominant signal abnormalities may be seen in the mesial temporal region, initially, and diffuse cortical-subcortical atrophy may appear on the long term. Certain authors think that there are different subgroups of clinical presentations among patients with GADA. Some data suggest that those with very high titers also have oligoclonal bands in their CSF and produce numerous additional auto-AB, suggesting polyautoimmune mechanisms, differing than those with lower titers, who may have less severe presentations. Pediatric data are scarce. To date, a few children only have been reported, most in the form of case reports or small series. With rare exceptions, detailed descriptions about work-up results are lacking: either GADA are only tested in serum samples, the evolution of the titers with time is wanting, or testing for additional auto-AB is not performed. In addition, EEG and imaging results are heterogeneous. Lin and coll. found positive GADA in 6 (35.3%) of their 17 children with encephalitis and SE. Interestingly, none of these children had diabetes. In that study, only one (2.5%) patient within the control group of 40 children with refractory epilepsy had positive GADA. GADA were not analyzed in the CSF. Like in adults, seizures in children with GADA seem to be particularly refractory to various therapeutic approaches, including immune ones. As an example, the patient we reported in 2011 had high levels of serum and CSF GADA, as well as intrathecal
AB production. She initially responded well to plasma exchanges, with cognitive improvement, diminished seizure frequency and parallel decrease in serum and CSF GADA levels. This improvement has unfortunately plateaued since, and she currently still has elevated serum GADA titers (although in much lower values than initially), severe developmental delay and daily seizures. These have remained refractory to various additional immune therapies, including interferon β.

Onconeural AB

A certain number of onconeural AB have been reported in patients suffering from various types of cancers and seizures. These are likely to be rare, particularly in children, though: in a large study of more than 20’000 serum samples screened for onconeural AB in France, 251 were positive, of which only 8 were children. Their targets include Hu (antineuronal nuclear antibody -ANNA-1-), Ri (ANNA-2), Ma2, Yo (Purkinje cell autoantibody 1), CV2/collapsin response mediator protein 5 antibody, and amphiphysin, among a few more. The clinical manifestations described in these patients often involve temporal lobe and limbic dysfunction (“limbic encephalitis”), which include personality changes, memory loss and seizures. Extratemporal manifestations have been more rarely reported, but recent imaging and EEG analyses of patients with anti-Hu AB indicate that these may be more frequent than suspected. In addition to the hippocampus and amygdala, abnormal MRI signals may be observed in the basal ganglia, the brainstem and the cerebellum. The discovery of such AB usually prompts active and careful search for neoplasms, such as small cell lung cancer in adults, or neuroblastoma in children. These associated diseases are likely to be rare in childhood, though. Like previously reported in a single case, 6 of the 8 pediatric patients with limbic encephalitis due to anti-Hu AB in the study by Honnorat, did not have cancer at the time of sampling.
5d.2) Neuronal surface targets

VgKC-complex proteins (LGI/Caspr2)

The voltage-gated potassium channel has been involved in autoimmune limbic encephalitides. Recent studies in adults showed that, more specifically, auto-AB target two subcomponents of the channel: leucin-rich, glioma inactivated 1 protein (LGI1), mainly involved in central nervous system manifestations, and contactin-associated protein 2 (Caspr2), more often observed in peripheral neurological symptoms, such as neuromyotonia (though also reported in children with encephalitis, or with new-onset seizures). The clinical symptoms engendered by their presence include faciobrachial dystonic seizures in close to 80% of adults, which seem specific enough to suggest the diagnosis even before other symptoms, such as cognitive dysfunction, appear. To our knowledge, such seizures have not been reported in childhood. EEG features are non-specific. Acute MRI may be normal, or show abnormal T2 and FLAIR signals in the hippocampus and amygdala, uni- or bilaterally, cortex, basal ganglia, as well as microbleeds; hippocampal sclerosis and brain atrophy may be seen on evolution. Like in adults, anti-VgKC AB have been mostly found in children with limbic encephalitis. Patients with anti-VgKC AB-related epileptic spasms or status epilepticus have also been reported. In a specific subgroup of 10 children with acute encephalitis, who presented with clusters of seizures and cognitive or behavioral troubles, 4 (40%) had positive anti-VgKC AB. The frequency of AB directed at the VgKC-complex, or LGI1 and CaspR2, in pediatric patients with epilepsy is unknown, however, and here again solid data are wanting in that group of age. Four (3.5%) of the 114 children with new-onset seizures reported by Suleiman and coll. had positive anti-VgKC AB, a similar finding to that of 5% reported in
adults with new-onset seizures. Immune therapies are usually very efficient in that situation. Spontaneous favorable evolution has also been reported in children. Parallel cancer treatment is warranted in those concerned.

**Glutamate receptors**

In addition to their observation in patients with Rasmussen encephalitis, auto-AB against various subunits of excitatory glutamate receptors have been identified in numerous adult and pediatric patients with signs of encephalitis or disorders in which seizures are observed. Overall, however, the various situations in which they were individualized were too heterogeneous to establish a clear and recognizable clinical correlate for each of them. One notable exception is represented by those directed against the NMDA-R, as described hereafter.

**NMDA receptors**

Auto-AB against various subunits of glutamergic NMDA-R have been described in association with seizures in adults and in children for more than a decade. The number of children reported has increased to the point that NMDA-R AB are now considered the second most frequent cause of autoimmune encephalitis in children, after acute demyelinating encephalomyelitis (ADEM). In addition, NMDA-R AB were more frequent than any viral etiology in patients enrolled in the California Encephalitis Project, in which patients with encephalitis of unclear cause are studied. An important step in the understanding and recognition of NMDA-R associated symptoms was taken when a homogeneous neuropsychiatric clinical presentation was described in an important number of adults in 2007. Likewise, a specific combination and evolution of symptoms in children with NMDA-R AB may suggest their presence. In a typical case, the patient presents with subacute
psychiatric and behavioral troubles, accompanied by rare seizures of non-specific semiology.\textsuperscript{128} Recent data suggest that children more frequently than adults present with neurological symptoms rather than psychiatric manifestations, initially.\textsuperscript{128} These symptoms are followed by the progressive appearance of a severe movement disorder, which includes oro-facial dyskinesias, dystonia, and choreoathetosis, that worsens and becomes continuous in the awake state. Neurovegetative instability, respiratory difficulties and sleep troubles may also be noted. Symptoms may start as early as in the first year of life.\textsuperscript{128} Cerebral imaging may either be normal, or show signs of a diffuse inflammatory process, such as patchy lesions on T2-weighted or FLAIR sequences in any region of the brain parenchyma, with or without contrast enhancement.\textsuperscript{98} Three distinct clinical radiological syndromes involving the white matter were recently identified in children with NMDA-R AB encephalitis.\textsuperscript{132} In many cases, the EEG correlate reflects a possible predominant involvement of subcortical structures, under the form of a generalized, slow, ample and monotonous background activity. Low-voltage fast rhythms unrelated to drug treatments may be superimposed in certain cases, and realize a so-called « extreme Delta-brush » pattern, also reported in certain children.\textsuperscript{128,133} Symptoms may last for months and may be life-threatening. The prognosis in children remains nevertheless favorable, and complete remission without sequelae is the rule.\textsuperscript{126,127,134} Spontaneous disappearance of symptoms has been reported. Current therapeutic recommendations suggest aggressive immunomodulatory management, and include the rapid use of cyclophosphamide (CTX) and monoclonal AB in case of initial failure with steroids, IvIg or plasma exchanges.\textsuperscript{128,134-136} Although likely to be rare in young girls, ovarian teratomas have been frequently reported in women with NMDA-R AB. If present, their surgical excision makes neurological symptoms disappear.
Folate receptors

Cerebral folate deficiency may be due to genetic or autoimmune processes. In the latter, auto-AB against the folate receptor are produced and prevent the transport of 5-methyltetrahydrofolate (5-MTHF), the active metabolite of folate, from blood to CSF by cells in the choroid plexus. As a consequence, the CSF level of 5-MTHF is low. The clinical and EEG findings of a few children with auto-AB against the folate receptor in their serum have been reported, including in one of them who was previously diagnosed with Alpers syndrome. Most of them had their initial events in their first two years of life, contrarily to those presenting with a similar clinical picture due to mutations on FOLR1, the gene that encodes the folate receptor, reported to start later in childhood. That feature apart, both forms of cerebral folate deficiency may be impossible to differentiate clinically. Seizures may be of various types and are typically resistant to classic anti-epileptic medications, but seizure control and overall prognosis may improve with the administration of folinic acid. EEG findings, when reported, usually show non-specific signs of a diffuse encephalopathy.

Calcium channels

Auto-AB against voltage-gated calcium channels are likely to be rare in patients with epilepsy: in the recent study by Brenner and coll., none of the 416 adult patients with epilepsy or new-onset seizures analyzed for autoimmune reactions had positive results for these AB. A group of researchers recently investigated the presence of auto-AB against the P/Q and T-type (involved in the genesis of absence seizures in children) calcium channels in 32 children and teenagers with absence seizures, as well as in 53 control patients with focal epilepsy, and 30 healthy subjects. Only one of
their control patients, also known for SLE, turned out to have AB directed against the P/Q type channel, whereas all of those with absences had negative results.\textsuperscript{142}

**GABA receptors**

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS. AB against its receptor subtypes GABA\(_A\) and GABA\(_B\) have been recently reported in relation with encephalitides and seizures.

**GABA\(_A\)**

AB against GABA\(_A\) receptors were recently reported at high levels in the serum, and were detected in the CSF, of 6 patients with a potentially treatable form of encephalitis, characterized by acute behavioral changes, seizures or status epilepticus with severe refractoriness to classic therapeutic approaches.\textsuperscript{143} Three of these 6 patients were under 18 years at the time of initial symptoms. One of them, aged 3 years at onset, also had serum and CSF GABA\(_B\) receptor AB and died in sepsis during status epilepticus; one was diagnosed with Hodgkin’s lymphoma 10 months before onset of neurological symptoms; she only had mild cognitive difficulties on follow-up and could return to school; the last one substantially recovered but had persistent seizures on follow-up. Patients with low serum titers of GABA\(_A\) receptor AB undetectable in the CSF were also described in that series, 3 of which were children with seizures. One of them had a subacute onset of seizures, presented actually in status epilepticus, and still had focal seizures on follow-up; another one had a subacute onset of abnormal movements, sleep disturbance, cognitive and psychiatric changes, and only brief seizures. Her work-up revealed the presence of AB against NMDA receptors in her serum and CSF, and she fully recovered; no follow-up data was available for the last one who presented with reduced verbal output and seizures, as well as GADA in her serum.
**GABA\textsubscript{B}**

Fifteen adult patients were described by Lancaster and coll. with GABA\textsubscript{B} receptor AB and subacute appearance of seizures, cognitive and behavioral disturbances\textsuperscript{144} One of them had presented with seizures in childhood, but whether the AB were present at this time is unknown. A few months later, Boronat and coll. reported 11 more adult patients with similar clinical features,\textsuperscript{145} most of which had associated small-cell lung cancer. A few more small patient series have been published since,\textsuperscript{146,147} but to our knowledge, these auto-AB have not been observed in children with seizures.

**Glycine receptors and Dipeptidyl-Pepitidase–Like Protein-6**

AB to glycine receptors (GLY-R) have been found in association with various types of epilepsies and progressive encephalomyelitis with rigidity and myoclonus (PERM) mainly in adults.\textsuperscript{115,148} In the series of Suleiman and coll., none of the 114 children with new-onset seizures tested in the 6 months that followed their initial ictal event had positive findings for GLY-R.\textsuperscript{104} Recently, a child with a two-year history of temper tantrums and focal seizures was found to have AB to GLY-R in his serum, but not in his CSF.\textsuperscript{149}

To our knowledge, this apparent rarity in children also applies to AB directed at Dipeptidyl-Pepitidase–Like Protein-6 (DPPX), an antigen involved in the function of Kv4.2 potassium channels, recently reported to cause acute encephalitis symptoms and diarrhea in 4 adults.\textsuperscript{150}

6. **Specific pediatric epilepsy syndromes in which auto-AB or dysimmune features have been reported or suspected** (Based on \textsuperscript{79}, with authorization)

A majority of children with seizures share certain clinical features that likely reflect a participation of the immune system in their disease. These include an increase (or, more rarely, and more surprisingly maybe, a decrease) in seizure frequency in
periods of infectious illnesses, or a favorable response to certain immunotherapeutic approaches. Some of the previously-described auto-AB, as well as additional auto-AB not primarily directed against CNS targets, elevated cytokines and other non-specific markers of an immune activation, have been found in children with certain well-delineated epilepsy syndromes, as described hereafter. Here again, detailed pathophysiological mechanisms remain to be understood, and the question as to whether these findings represent a cause or a consequence of seizure activity remains open.

6a. West Syndrome

West syndrome is characterized by the association of epileptic spasms, hypsarhythmia, and developmental delay or regression. It typically appears in children aged less than 1 year, and has multiple potential underlying causes, including structural lesions and genetic defects. Montelli and coll. showed that certain children with West syndrome of various etiologies had a global dysfunction of their immune system, which included unusually elevated IgG and IgM levels, and an abnormal cellular response. More recently, anti-VgKC complex AB were demonstrated in the serum of a 13-month old child with West syndrome, whose work-up also revealed an oligoclonal distribution in his blood and CSF. This patient had a normal MRI, and no additional auto-AB were found. One of the children mentioned above and found to have auto-AB directed against the folate receptor, presented with epileptic spasms and hypsarhythmia at 6.5 months. Finally, increased serum levels of anti-convulsive IL-1R antagonist was demonstrated after spasm control in children with West syndrome. Contrarily, none of the children with new-onset seizures and positive auto-AB reported by Suleiman and coll. (Suleiman 2013) had
West syndrome,\textsuperscript{104} and CSF interleukin 6 (IL-6) levels were normal in the 12 patients with West syndrome reported by Tekgul and coll. in another study.\textsuperscript{153}

6b. Landau-Kleffner Syndrome

Landau-Kleffner syndrome (LKS) is a developmental disease of the CNS characterized by an acquired aphasia related to the presence of continuous spike-waves during non-REM sleep in bilateral temporal regions.\textsuperscript{154} It typically develops in previously normal school-age children, who progressively lose their ability to understand and interpret the sounds they hear (auditory agnosia), in the presence of preserved sensory capacities. Seizures are usually rare and easy-to-treat. Depending on severity and localization of EEG abnormalities, the disease may cause additional cognitive and behavioral difficulties, such as expressive speech troubles, attention deficit and hyperactivity.\textsuperscript{155} In spite of recent descriptions of certain genetic defects in a subset of children with LKS,\textsuperscript{156-158} its pathophysiology remains unclear, more than 50 years after its initial description. Although potentially very difficult-to-treat, EEG abnormalities may respond well to immunotherapeutic approaches that include multiple schemes of various oral or intravenous steroids, including at high doses and for a prolonged period of time.\textsuperscript{159} Connolly and coll. reported more frequent (although not significantly) anti-endothelium AB in patients with LKS than in children with other neurological diseases, such as autism.\textsuperscript{160,161} AB against various other parts of the CNS and a possible role for autoimmunity have also been reported by others in rare children with LKS,\textsuperscript{162,163} but the rare histological descriptions available have not allowed firm confirmation of an immune component to that syndrome.\textsuperscript{164}

6c. Acute encephalopathy with immune-mediated status epilepticus
Acute encephalopathy with immune-mediated status epilepticus (AEIMSE) is a group of acute encephalopathies that present in childhood with status epilepticus episodes of suspected immune origin. These include idiopathic hemiconvulsion-hemiplegia syndrome (IHHS), formerly known as hemiconvulsion-hemiplegia-epilepsy (HHE), as well as febrile infection-related epilepsy syndrome (FIRES), formerly named acute encephalitis with refractory, repetitive partial seizures (AERRPS); new-onset refractory status epilepticus (NORSE), or devastating encephalopathy in school-age children (DESC). The most recently described FIRES, is characterized by acute, refractory, repeated seizures or status epilepticus that appear in close relation with a non-specific febrile disease. Seizures may last for weeks. Most who survive this initial critical phase develop a difficult-to-treat focal epilepsy and severe cognitive sequelae. Infectious causes have never been demonstrated; various mitochondrial, genetic or dysimmune hypotheses have been raised. In rare individual patients only, auto-AB against GAD, VgKC, GLUR-3, GLUR- δ2, smooth muscle, cardiolipin, neuropil, cellular nucleus, or beta-2 glycoprotein, were demonstrated in their serum or CSF. Available histopathological studies are rare and do not show definite signs of CNS inflammation. Early immunotherapy may nevertheless improve the prognosis of FIRES, otherwise considered as ominous.

6d. Dravet syndrome

Dravet syndrome (DS) is a severe genetic epilepsy that typically starts in the first year of life with febrile, repeated or prolonged hemifocal seizures in previously normal children. Mutations on SCN1A, the gene that encodes the alpha-subunit of the type 1 sodium channel, are found in 70-80% of patients with DS. Additional genes have been involved in small subgroups of patients with DS. High seizure
sensitivity to fever is characteristic of the syndrome. Additional seizure types are rapidly observed, and include myoclonias, generalized tonic-clonic seizures, absences or dyscognitive seizures, most of which are difficult-to-treat. Repeated status epilepticus episodes are the rule. Severe intellectual disability appears in the second year of life. Previously considered a definite epileptic encephalopathy, in which seizures were considered directly responsible for the intellectual decline, recent observations of persistent difficulties despite good seizure and EEG control in patients with DS have questioned that impression. Detailed histopathological findings from autopsies or surgical biopsies, including results of specific immune markers, were recently reported in 22 adult patients with DS, and compared to controls (such as children with DS, or adults with other neurological disorders). Important features included connexin 43 (Cx43-) and glial fibrillary acidic protein (GFAP-) immunopositive cells noted in the cortex and hippocampus of patients with DS. These features were not significantly different from control patients, however.\textsuperscript{182} Whether certain patients with clinical DS but negative genetic investigations actually suffer from a dysimmune CNS disorder remains to be demonstrated.

7. Therapeutic approaches and prognosis

Various drugs and molecules with anti-inflammatory or immunomodulatory properties have been shown to decrease the occurrence of additional seizures in acute clinical or experimental situations, or to prevent kindling and epilepsy development in animals.\textsuperscript{58,183-190} Their mechanisms of action are summarized in Table 2. It is to note that contradictory data exist for some of these findings, e.g. for certain of the “classic” AEDs, like valproate, shown to produce strong microglial activation in an experimental model of CNS inflammation.\textsuperscript{191} Clinically, numerous observational studies, case reports and patient series have clearly demonstrated the potential
benefits of immunotherapeutic approaches in various established epileptic conditions. A favorable clinical response to such treatments has been sometimes considered a diagnostic criterion for an autoimmune component to the disease. As a general rule, situations in which neuronal auto-AB are found and target surface antigens (NMDA-R or VgKC-complex proteins) have a much higher response rate than those in which antigens are intracellular, such as GAD.\textsuperscript{192} Potential immune approaches for children in various epileptic conditions include steroids,\textsuperscript{193,194} IVlg,\textsuperscript{195-197} plasma exchanges,\textsuperscript{198,199} rituximab (RTX), CTX, and « alternative » approaches such as the ketogenic diet (which anti-inflammatory and neuroprotective properties are likely to be major players in its mechanisms of action).\textsuperscript{57,165,200-202} Notably, some of these treatments have been considered as first-line therapeutic options in certain epilepsy syndromes with refractory seizures, even in the absence of formally identified inflammatory parameters, auto-AB or immune dysfunction. The most illustrative of these situations is West syndrome, for which ACTH or prednisone were (and still are) considered as primary therapeutic choices whatever the underlying cause,\textsuperscript{203,204} and long before the involvement of immunity in epilepsy had been suspected. Interestingly, many “classic” anti-epileptic drugs and therapies also possess anti-inflammatory properties,\textsuperscript{189} which include inhibition of the nuclear transcription factor NF-kB by valproic acid, propofol, thiopental, or ketamine; protection of the blood-brain-barrier by ketogenic diet, vagal nerve stimulation or hypothermia; and inhibition of the T-cell mediated production of interferon-\textgamma by diazepam.\textsuperscript{57} For certain authors, these characteristics may explain the efficacy of certain AEDs on additional conditions in which an important inflammatory component is involved, such as pain.\textsuperscript{57} Others, such as carbamazepine, may contrarily exhibit proinflammatory effects.\textsuperscript{57} Whether this characteristic may explain the potential of
that drug to exacerbate seizures in certain generalized epilepsies has to be considered.

Significant data from randomized prospective studies and long-term follow-up in large patient series remain scarce, and no consensus has been reached yet as to which patients with epilepsy should be treated with immunomodulating drugs, and how, precisely. Two meta-analyses on the use of intravenous immunoglobulins or immunomodulatory interventions overall in epilepsy concluded that, based on available data, their efficacy could not be demonstrated.\textsuperscript{205,206} Thus, the question as to whether anti-inflammatory or immunomodulating therapies should be added to classic AEDs in autoimmune, or even in all types of epilepsies, remains open. In a recent study, Irani and coll. reported a rapid decrease and progressive total disappearance of seizures in 9 of 10 patients with AB against the VgKC-complex treated with steroids in addition to the initial AEDs.\textsuperscript{116}

Similarly, Toledano and coll. selected 29 of 110 patients followed at their neuroimmunology clinic who presented with seizures as a major complaint. Children as young as 2 years were included. These patients were suspected of having seizures of autoimmune etiology, based on the presence of at least one neural autoantibody, personal or family history or physical stigmata of autoimmune disorders, and frequent or refractory seizures. Treatment with intravenous methylprednisolone for 6 to 12 weeks and IvIg (alone or in combination), was administered. Eighteen patients responded with a decrease in seizure frequency, which in the majority of cases was sustained. The study had methodological weaknesses, though, and only brought class IV evidence that these therapeutic options improved seizure control.\textsuperscript{207}

\textbf{8. Open questions and future perspectives}
It is now clear that inflammation and autoimmunity play an important role in childhood seizures and epilepsies. On the one hand, these reactions can be the cause of seizures, like when auto-antibodies against various CNS targets involved in neuronal activation are produced. On the other hand, various CNS and peripheral immune responses are activated after seizures. The latter mechanisms are encompassed in the recently described concept of “neurogenic neuroinflammation”, which consequences may either be the activation of an anti-inflammatory cascade and homeostatic mechanisms, with subsequent neuroprotection and interruption of seizures; or the perpetuation of a maladaptive and neurotoxic response at the basis of further epilepsy genesis. Why this physiological initial response progresses to dysfunctional processes in certain individuals is unknown. In fact, many of the questions raised by Vezzani and Rüegg in 2011 remain open. In particular, the clinical significance of many discoveries in the field, and their applicability to daily practice, are still to be understood. Available data allow proposing partial answers to three important patient-oriented questions, as follows.

8a. When should one clinically suspect a child with seizures of immune etiology? (Figure 3)

Suleiman and coll. proposed a flowchart to approach children with suspected autoimmune seizures. Likewise, Bien proposed a detailed table of clinical and paraclinical features that should prompt the search for auto-AB in patients with epilepsy. From a more general standpoint, we propose that immune function analyses should be specifically considered in children if three of the four following criteria are present: a. Acute or subacute onset of seizures, repeated in a short time interval; b. Additional clinical signs suggest diffuse CNS involvement (such as psychiatric or behavioral troubles, disturbance of consciousness, abnormal
movements, or sleep problems); c. The clinical presentation does not orient to well-circumscribed and rapidly identifiable epilepsy syndromes (such as epilepsy with centro-temporal spikes, Panayiotopoulos syndrome), and structural lesions, infectious, metabolic or toxic diseases are a priori excluded by history; d. The clinical presentation clearly orients to a syndrome associated with the presence of specific autoantibodies (e.g. subacute psychiatric disturbances, seizures and orofacial dyskinesias associated with NMDA-R AB; or faciobrachial dystonic seizures and AB against VgKC complex).

8b. How should a child with seizures of suspected immune etiology be evaluated? (Figure 3)

Targeted blood analyses for the most likely auto-AB according to clinical presentation, or the use of “biochips” (mosaics of cells displaying various antigens), and a cerebral MRI with T2 and FLAIR sequences, should be the minimal procedures performed initially in children with seizures of suspected immune etiology. A lumbar puncture should then be performed in those whose initial work-up adds suggestive elements of CNS inflammation to the clinical presentation, to look for increased protein or cell count, oligoclonal bands and CNS production of auto-AB that may have been found in their blood in parallel. An extended blood work-up (including complement system proteins, sedimentation rate, antinuclear and anti-DNA AB, etc…) for systemic autoimmune diseases should also be considered, especially in those with multi-organ involvement. A standard EEG may bring additional clues in specific situations, like when NMDA-R encephalitis is suspected and the pattern of extreme delta brush described above may be present. Tumor search should be performed according to the potential finding of auto-AB specifically associated with certain malignancies.
8c. How should a child with seizures of demonstrated (or strongly suspected) immune etiology be treated?

The “classic” schemes used in other autoimmune or dysimmune neurological diseases may also be applied to seizures and epilepsy of immune etiology. As suggested by various authors,\textsuperscript{192,210,211} the addition to AEDs of intravenous methylprednisolone at 30 mg/kg/day for 3-5 days, followed by oral prednisone or prednisolone at 1-2 mg/kg/day for 2-4 weeks and slow taper seems a reasonable initial (“first-line”) approach for all patients, and should be considered as soon as suspicion of immune etiology arises, be it in chronic or in acute situations. As specifically demonstrated in certain conditions, such as NMDA-R encephalitis,\textsuperscript{135,136,211} “aggressive” treatment including a minimum of 4-10 plasma exchanges,\textsuperscript{192} followed by IVIg at 2 g/kg divided by 2-5 doses (or, alternatively, IVIg followed by plasma exchanges after a minimal two-week interval),\textsuperscript{212} and various combinations of immunosuppressive drugs (including intravenous RTX at 375 mg/m\textsuperscript{2} every week, 2-4 times; and intravenous CTX at 750 mg/m\textsuperscript{2}, monthly)\textsuperscript{192} should be rapidly envisaged after failure of steroids.

One must take into account, however, that available clinical data on immune treatment approaches are weak, and that evidence regarding the specific aspects of success rates, complications and need for continued therapy is lacking. In addition, therapeutic approaches specifically tailored to suspected underlying mechanisms of disease do not currently exist.

Among many more, important questions that remain open include the understanding of the precise timing and sequence of elements of the immune response to seizures; the detection of reliable diagnostic biomarkers of CNS inflammation in children with
epilepsies; the identification of specific clinical, radiological and electrophysiological features that may allow early suspicion; and the development of optimal therapeutic strategies and molecules targeted against the various inflammatory mediators described above, through prospective controlled studies. These points offer fascinating basic and clinical research topics, and raise hope for the development of concrete therapeutic perspectives for an important number of children in a close future.
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Table 1: Neuronal antigens associated with seizures in children (Adapted from Bien, [http://www.mara.de/start.html](http://www.mara.de/start.html))

<table>
<thead>
<tr>
<th>Intracellular antigens</th>
<th>Surface antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VGKC complex</td>
</tr>
<tr>
<td>Onco-neural GAD</td>
<td>LGI1 CaspR2</td>
</tr>
<tr>
<td>Reported in children</td>
<td></td>
</tr>
<tr>
<td>Suggestive features</td>
<td>Type-1 diabetes</td>
</tr>
<tr>
<td>Prognosis in children</td>
<td>Unfavorable</td>
</tr>
</tbody>
</table>

Abbreviations: CaspR2: contactin-associated protein; GABA: gamma aminobutyric acid; GAD: glutamic acid decarboxylase; LGI: Leucin-rich glioma inactivated protein; NMDA: N-methyl-D-aspartate; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; R: receptor; VGKC: voltage gated potassium channels
Table 2: Examples of drugs and treatments with anti-inflammatory or immunomodulatory properties, that have shown experimental or clinical efficacy in seizure treatment or prevention of epilepsy development

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Examples of drugs / treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBB homeostasis control</td>
<td>Steroids, Ketogenic diet, Hypothermia, Vagal nerve stimulation, Erythropoietin, Magnesium sulfate, Rapamycin</td>
</tr>
<tr>
<td>Decreased leukocyte adhesion to BBB</td>
<td>Natalizumab, Steroids, IL-R antagonists (e.g. anakinra), Integrin α-4 specific monoclonal AB</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td></td>
</tr>
<tr>
<td>• Inhibition of auto-AB production</td>
<td>Steroids, Cyclophosphamide, Rituximab</td>
</tr>
<tr>
<td>• Inhibition of T-cell response</td>
<td>Tacrolimus, Diazepam</td>
</tr>
<tr>
<td>Inhibition of cytokine production</td>
<td>Minozac, ICE-inhibitor (e.g. VX-765), Levetiracetam</td>
</tr>
<tr>
<td>• Via NF-κB inhibition</td>
<td>Valproate, Propofol, Thiopental, Ketamine</td>
</tr>
<tr>
<td>Removal of auto-AB</td>
<td>IvIg, Plasma exchanges</td>
</tr>
<tr>
<td>PTGS2 inhibition</td>
<td>NSAIDs: Celecoxib, Parecoxib</td>
</tr>
<tr>
<td>Microglia inactivation</td>
<td>Erythropoietin</td>
</tr>
</tbody>
</table>

Abbreviations: AB: antibody; BBB: blood-brain barrier; ICE: Interleukin converting enzyme; IL: interleukin; IvIg: intravenous immunoglobulins; NF: nuclear transcription factor; NSAID: non-steroidal anti-inflammatory drug; PTGS2: prostaglandin G/H synthase 2 (formerly COX-2); R: receptor
3. NEURONAL FUNCTIONAL CHANGES

Increased expression of IL-1R1, intracellular kinase activation, inhibition of the glutamate reuptake, synaptic reorganization, ion channel dysfunction,…

2. RELEASE OF INFLAMMATORY MEDIATORS

Cytokines, chemokines, COX2 pathway and complement proteins, CAMs, danger signals,…

1. INITIAL INJURY

**PERIPHERY**: infection, autoimmune disease,…

**CNS**: seizure, infection, vascular disease,…

**Inflammatory response**

**BBB breakdown**

**EPILEPTOGENESIS**

- Lower secure threshold
- Increased neuronal excitability
- Increased neuronal excitability
Figure 3

Suggestive features for seizures of immune etiology (3 must be present)
- Acute or subacute onset
- Signs of diffuse CNS involvement
- Identifiable epilepsy syndromes or other structural/metabolic causes excluded by history
- Signs and symptoms consistent with specific auto-Ab

Initial work-up
- Brain MRI (T2 and FLAIR)
- Blood auto-Ab panel

Specific work-up to be considered, if initial results further suggest CNS inflammation
- EEG
- Lumbar puncture
  - cell count
  - protein level
  - oligoclonal distribution
  - auto-Ab panel
- Extended blood analyses for systemic autoimmune diseases
- Tumor search
Legends to figures

Figure 1
PubMed search: "antibodies and epilepsy", number of publications per year (1955-2014)

Figure 2
Molecular mechanisms involved in the immune cascade related to seizures and epilepsy

Figure 3
Children with seizures of suspected immune etiology: suggestive clinical features and proposed stepwise diagnostic approach