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EXPERT
REVIEWSEpileptic seizures as a
manifestation of cow's milk
allergy: a studied relationship
and description of our
pediatric experience*Expert Rev. Clin. Immunol.* 10(12), 000–000 (2014)

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Adverse reactions after ingestion of cow's milk proteins can occur at any age, from birth and even amongst exclusively breast-fed infants, although not all of these are hypersensitivity reactions. The most common presentations related to cow's milk protein allergy are skin reactions, failure to thrive, anaphylaxis as well as gastrointestinal and respiratory disorders. In addition, several cases of cow's milk protein allergy in the literature have documented neurological involvement, manifesting with convulsive seizures in children. This may be due to CNS spread of a peripheral inflammatory response. Furthermore, there is evidence that pro-inflammatory cytokines are responsible for disrupting the blood–brain barrier, causing focal CNS inflammation thereby triggering seizures, although further studies are needed to clarify the pathogenic relationship between atopy and its neurological manifestations. This review aims to analyze current published data on the link between cow's milk protein allergy and epileptic events, highlighting scientific evidence for any potential pathogenic mechanism and describing our clinical experience in pediatrics.

KEYWORDS: atypical clinical features • CNS inflammation • cow's milk allergy • pro-inflammatory cytokines • seizures

The global prevalence of food allergy is estimated to range between 220 and 520 million people [1]. Adverse reactions after ingestion of cow's milk proteins can occur at any age from birth, and even among exclusively breast-fed infants, although not all of these are hypersensitivity reactions [1].

In general, food allergy is more frequent in childhood than in adulthood; and according to a recent Japanese multicentre study, the prevalence of cow's milk protein allergy (CMA) is 0.21% in newborns and 0.35% among extremely premature babies (birth weight <1000 g) [2].

In a large European survey [3], in which more than 44,000 individuals were contacted, data of 8000 children living in Austria, Belgium, Denmark, Finland, Germany, Greece, Italy, Poland, Slovenia and Switzerland was obtained. For each telephone contact, a multiple choice questionnaire to assess CMA epidemiology, clinical signs and symptoms and chosen treatment was

submitted to each individual of the family. The estimated prevalence of CMA in this study was 4.7%, with the highest rate in children between 2 and 3 years of age (7.2%). Wide national differences were noted, with prevalence rates ranging from 13.8% in Greece to 52.3% in Finland. In addition, milk was the most frequently reported offending food in children (38.5% of atopic cases) and the second most frequently implicated in adulthood (26%). Furthermore, the most frequent allergic manifestations of CMA were: skin reactions (70% of cases), gastrointestinal (25%) and respiratory (20%) symptoms. In 70% of cases, a therapeutic treatment was needed. However, it is important to differentiate CMA from cow's milk protein intolerance, which is a distinct entity with some similarities to CMA. This is present in the western world with an incidence around 2–5% among newborns [4]. It is fundamental for pediatricians to differentiate between cow's milk

50 protein intolerance and CMA to diagnose those forms of intolerance usually evolving towards CMA, exposing these patients to a high risk of anaphylaxis [5].

55 CMA typically presents with gastrointestinal symptoms, such as vomiting, diarrhea and/or constipation as well as gastroesophageal-reflux. However, 'atypical' symptoms, involving the CNS and peripheral nervous systems, have been recently identified. These symptoms can occur in both IgE- and non-IgE-mediated CMA, as an extension of the diffused, multiorgan inflammatory response to CMA. Furthermore, recent research 60 attempts to explain the association between CMA and seizures have been led by Riazi *et al.*, who showed a possible role for systemic inflammation in increasing neuronal excitability and leading to greater seizure susceptibility [6].

65 The aim of our review is therefore to analyze the current literature on the link between CMA and epileptic events, highlighting the evidence that could explain a pathogenic link between the two diseases in conjunction with describing our experience in pediatrics.

Methods

70 Articles reporting evidence on the association between food allergy, particularly CMA, and neurological disease, with or without statistical meta-analysis, were selected. Those reporting evidence of one or more other non-allergic (comorbid) disorders were considered (outcome). Studies that assessed the relationship 75 between atopic diseases with non-specific symptoms, such as psychosocial distress or sleeping problems, were excluded.

Given the lack of an electronic database that contains all publications of all medical journals and that a restriction of only one database could be associated with a systematic bias, it was necessary to combine multiple databases for a comprehensive literature search. For this reason, an electronic literature search was carried out in MEDLINE via PubMed interface, SCOPUS, Google Scholar, the Cochrane Library for all articles published from inception to October 2013. Database-specific search strings were 80 developed and included search terms describing food allergy and/or CMA (population/exposure) and neurological signs or symptoms (study design/description of cases). A combination of medical subject headings and keywords was used.

85 Titles and abstracts of identified papers were screened by two independent reviewers to determine whether they met the eligibility criteria of interest to develop our review. Subsequently, full texts of the remaining articles were independently retrieved by the two reviewers for eligibility.

Peripheral inflammation as trigger of seizures: a link between the gastrointestinal & the neurological system

95 It has been recently demonstrated that peripheral inflammation reflects a similar inflammatory state in the brain [6]. The CNS inflammation is characterized by microglial activation with an increase of pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6 [6]. Recent attempts have been made to study the link 100 between peripheral and central inflammation, with evidence that cytokines can directly influence *in vitro* synaptic functions

and neural properties [7–12] or can alter transmitter biosynthetic pathways [13,14]. Nevertheless, the pathogenic mechanism that links peripheral and central inflammation is still poorly understood, despite inflammation being recognized as an important 105 trigger for seizures, it is pertinent to investigate its potential role in mediating this pathogenic relationship. However, despite the link between CNS inflammation and seizures being well established, poor data are available on the link between peripheral inflammation and seizures as well as knowledge of the relationship between peripheral inflammation and neuronal excitability (TABLES 1 & 2) [15,16].

Because the hippocampus is fundamental for the onset and propagation of seizures, Riazi *et al.* [6] have shown how peripheral inflammation can alter hippocampal neuronal excitability studying a murine model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. TNBS is a Th1-cell-mediated model of inflammatory bowel disease that during acute phases is associated with activation of the humoral immune system, causing a localized 115 inflammatory colitis [17–19]. Furthermore, Riazi *et al.* identified a model of microglial-dependent, TNF-mediated increase in CNS excitability that may be considered a potential link between peripheral and central inflammation. The clinical evidence of this relationship was demonstrated by the same authors through a reversible increase in seizure susceptibility, directly correlating with the grade of severity of inflammation and hippocampal electrophysiological recordings of inflamed mice. These findings revealed increased excitability and epileptiform burst discharges to bath application of potassium channel blocker 4-AP, as expressed by increased excitability of neuronal networks in the hippocampus following peripheral inflammation. These findings, therefore, suggest that the production of TNF- α occurs within the brain during peripheral inflammation, increasing seizure susceptibility. Moreover, increased TNF-signaling in the CNS as well as microglial activation appeared 125 sufficient for changes in seizure susceptibility. Furthermore, the authors also noted that colonic inflammation lowered the threshold for the induction of IV pentylenetetrazole-induced seizures. A possible pathogenic mechanism was suggested which alters pentylenetetrazole metabolism due to a disruption of the blood–brain barrier (BBB) that occurred in this inflammatory model [20], eventually resulting in an increased uptake of pentylenetetrazole within the brain. Therefore, not only can this model potentially provide a better understanding of peripheral inflammation but also how this may present with features that involve the CNS, giving new insights 135 into co-morbidities resulting from altered CNS function in the context of peripheral inflammatory diseases [6].

The immune mechanism of epileptogenesis as a consequence of peripheral inflammation has centered upon the role of microglia. Microglial activation as a consequence of peripheral inflammation was confirmed by the increased number of microglial cells in the hippocampus and adjacent areas [21–23]. In keeping with this observation, the authors showed an increased production of hippocampal TNF- α , despite low IL-1 levels, explaining that these findings were likely related to the colonic inflammatory model, which at the height of colic inflammation is associated with a downregulation of IL-1 synthesis in comparison with 145

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Table 1. Clinical studies on the evidence of a relationship between systemic gastrointestinal inflammation of various nature and seizures.

Type of study	Population	Population description	Analytic features	Results	Ref.
Prospective observational studies	Pediatric population	Reference population of 39,000 patients	Incidence study	Fourteen cases of GI associated with seizures. The authors estimated an incidence of gastroenteritis-related seizures of 1 out of 10,000 children for afebrile seizures associated with gastroenteritis, with an earlier onset of symptoms with respect to the febrile seizures, and at a slightly higher age	[28]
Retrospective observational study	Pediatric population	Authors studied the electrophysiological features of these gastroenteritis-associated seizures using EEG on 25 pediatric patients	Observational study	Among the observed EEG, 32% were affected by focal, tonic or clonic seizures, 12% of focal with secondary generalization, 36% of generalized tonic-clonic seizures and 20% consisted of staring only (absence-like) with no motor components, 52% of patients had clusters of seizures and 48% had isolated seizures. Interictal EEGs and brain imaging were normal for all patients. No patients required treatment with antiepileptic drugs	[29]
Retrospective cohort study	Pediatric population	323 children admitted to pediatric emergency room for seizures	Observational study	Among the studied population, 247 (76%) had febrile seizure, 37 (12%) had unprovoked seizures and 39 (12%) had non-febrile illness seizures. Children with non-febrile illness seizures were more likely than children with febrile seizures to have diarrheal illnesses accompanying their seizure (44 vs 16%; $p = 0.001$). Frequency of cough, rhinorrhea and rash did not differ significantly between children with febrile and non-febrile illness seizures	[31]
Retrospective observational study	Pediatric population	400 asthmatic patients and 201 affected by idiopathic epilepsy	Prevalence study	Prevalence of 0.75% of idiopathic epilepsy among the 400 cases of asthma and prevalence of 0.79 and 0.73% of epilepsy in mild and moderate-to-severe asthma, respectively. Similar prevalence in general population	[43]
Case report	Pediatric population	3 children affected by cryptogenetic partial epilepsy	Observational study	Reduction of SCORAD in children with SPT ⁺ positive for CMA; Improvement of eczema;	[44]
Retrospective observational study	Pediatric population and their relatives	72 epileptic children and 202 controls	Prevalence study	Significant statistical higher rate of eczema in epileptic patient's mothers and rhinitis in their siblings with respect to controls	[45]
Perspective observational study	Pediatric population	56 children affected by Rolandic epilepsy	Observational study and therapeutic intervention	Non-allergic children displayed complete clinical and EEG remission in 29 out of 39 cases (74%). The cow's milk-free diet made it possible to obtain clinical remission in 17 out of 20 cases (80%) of the allergic group with normalization of the EEG. Less adverse events in the patients with no anticonvulsant therapy	[46]

CMA: Cow's milk protein allergy; GI: Gastrointestinal Inflammation.

Table 2. Experimental studies on etiopathogenic hypothesis on peripheral and central inflammation triggering seizures.

Type of study	Population	Population description	Analytic features	Results	Ref.
Prospective observational study	Animal model	Male rats treated with TNBS and lipopolysaccharide during active colitis; seven rats as control group	Experimental study	At the peak of colitis, treated rats showed a disruption of circadian body temperature rhythm, as daytime fever followed by nighttime hypothermia. In response to lipopolysaccharide, treated animals had a significantly reduced fever and low plasma levels of IL-6 and TNF- α , while controls displayed a characteristic fever. During the phase of reactivation of colitis, treated animals did not show any fever or exhibit increased IL-6 and TNF- α after lipopolysaccharide injection	[18]
Prospective observational study	Animal model	Adult rats in which authors administered an intracolonic administration of TNBS to initiate a T helper-1 cell-mediated model of inflammatory bowel disease	Experimental study	Authors identified a microglia-dependent TNF α -mediated increase in CNS excitability associated with a phenotypical expression of increased frequency of seizures. The magnitude of the seizure susceptibility change was slightly weaker than what was observed after TNBS-induced inflammation. This phenomenon could be related to the incomplete diffusion to all brain areas of exogenous TNF- α such as amygdala and endorhinal cortex, both distant from the ventricles and involved in seizure generation	[19]
Prospective observational study	Animal model	FcRgamma-deficient mice (group 1) and in FcRgamma present mice (wild type B6) (group 2) in which hippocampal pyramidal cell death was induced by kainic acid (KA)	Experimental study	No activated microglial cells were detected 24 h after the KA injection in group 1, whereas many activated microglial cells were present in group 2. After 16 h the KA injection, in group 2 the production of nitrotyrosine as well as of the inducible nitric oxide synthase and cyclooxygenase-2 proteins. By contrast, the magnitude of oxidative stress was mild in group 1. Co-injection of a neutralizing antibody against FcRgammaRII and FcRgammaRIII with KA abolished pyramidal cell death and microglial activation	[57]
Prospective observational study	Adult population and animal model	Authors monitored the onset of seizures in patients undergoing osmotic disruption of BBB followed by intra-arterial chemotherapy to treat primary brain lymphomas and in naive pigs. Procedures were performed under barbiturate anesthesia	Observational and experimental study	Focal motor seizures occurred immediately after BBB disruption in 25% of procedures and originated contralateral to the disrupted hemisphere. In a porcine model identical procedures generated identical results, and sudden behavioral and EEG seizures correlated with successful BBB disruption	[69]
Prospective observational study	Animal model	Fifteen laboratory rats	Experimental study	Authors recorded EEG before, during and after hypertonic mannitol injection into the internal carotid artery. Rats' BBB disruption was evaluated by gross anatomy or fluorescent microscopy. This disruption occurred in 20% of the rats and only under this circumstance acute EEG changes, recorded as brief EEG slowing, followed by a gradual increase in activity, were detected	[70]
Prospective observational study	Animal model	Adult rats wild-type and perforin-deficient mice. Splenectomy was performed before pilocarpine injection	Experimental study	Splenectomy significantly reduced seizure-associated mortality. Histologic analysis of the spleens of rats exposed to pilocarpine revealed a significant increase in CD3 ⁺ and CD8 ⁺ T cells. Onset of status epilepticus and mortality were significantly decreased in perforin-deficient mice. The BBB disruption damage was decreased in the perforin-deficient pilocarpine-treated mice	[74]

Table 2. Experimental studies on etiopathogenic hypothesis on peripheral and central inflammation triggering seizures (cont.).

Type of study	Population	Population description	Analytic features	Results	Ref.
Prospective observational study	Animal model	Adult rats in which muscarinic receptor antagonist (atropine) and agonist (oxotremorine) were administered chronically through miniosmotic pumps	Experimental study	Oxotremorine stimulated, atropine inhibited the antibody and T-cell proliferative responses. Atropine suppressed the turpentine-induced leukocytic infiltration and tissue injury, and inhibited chemotaxis of leukocytes toward neutrophil and monocyte/lymphocyte chemoattractants	[75]
Prospective observational study	Animal model	Twelve- to 14-week-old male wild-type and knock-out mice	Experimental study	Using immunohistochemistry for CD45 ⁺ and CD3 ⁺ T-lymphocytes, authors showed microglial activation and infiltration of leukocytes in sclerotic tissue from patients with mesial temporal lobe epilepsy, as well as in a model of TLE (intrahippocampal KA injection), characterized by spontaneous, nonconvulsive focal seizures	[77]

TNF- α secretion. These findings have been supported by literature implicating pro-inflammatory cytokines in seizure activity and epilepsy [24–27], with a predominant role for IL-1 β as a pro-convulsing cytokine and IL-1 receptor antagonist (IL-1Ra), mainly playing an anticonvulsant role. However, Riaz *et al.* show in their studies that IL-1Ra did not have any significant effect on epileptogenesis whereas the blocking of central TNF- α was alone effective as an anticonvulsant mechanism. The authors therefore had good evidence to conclude that TNF- α was responsible for microglial activation and consequently triggering seizures. According to this evidence, it might be expected that chronic exposure to CNS TNF- α might induce a decrease in seizure threshold. On the contrary, Riaz *et al.* [6] found that a 4-day TNF- α infusion into the lateral ventricle of the mouse models (a time sufficient to induce maximum colitis and seizure susceptibility) caused a significant decrease in seizure threshold. The magnitude of the seizure susceptibility change was slightly weaker than what was observed after TNBS-induced inflammation. This phenomenon could be related to the incomplete diffusion of exogenous TNF- α to all brain areas, such as the amygdala and the endorhinal cortex, both distant from the ventricles and involved in seizure generation.

This link between peripheral and CNS inflammation, and in particular gastrointestinal inflammation and seizures, through the involvement of TNF- α and IL-1 β cytokines, may explain the neurological involvement in clinical gastroenteritis recently described in the literature. Riaz *et al.* showed this basic link using experimental evidence whereas other researchers have led studies in the clinical field to support this hypothesis. Recently, Maseri *et al.* [28] studied the incidence of gastroenteritis-related seizures. The authors prospectively collected data on all cases of pediatric seizures associated with gastroenteritis without electrolyte abnormalities, over a period of 2 years. They then divided the included patients in two groups, according to the presence or absence of fever during the convulsive episode. The authors studied 14 cases, from a reference population of 39,000 patients, characterized by a homogeneous phenomenological presentation of the convulsive episodes. They found that the onset of convulsive

episodes was earlier in those patients affected by febrile gastroenteritis and that in 13 out of 14 cases the ictal phenomenology was consistent with generalized convulsive episodes of which seven were tonic-clonic seizures, five generalized hypertonia and one generalized hypotonia. Only in one case, the convulsive episode presented without fever, manifesting as a partial seizure with oculo-facial deviation towards the left side, along with clonus of the patient's upper lip. The authors estimated an incidence of gastroenteritis-related-seizures of 1 out of 10,000 children for afebrile seizures associated with gastroenteritis, with an earlier onset of symptoms with respect to those patients affected by febrile seizures. Moreover, in the studied patients, an EEG was performed and in most cases a normal baseline activity was found, while non-specific and transitory abnormalities were found in five cases. The seizure episodes presented with recurrent features in 9 out of 14 patients, while in seven patients these episodes lasted up to 4 min and only in one patient more than 20 min. Fifty percent of patients were treated with rectal Diazepam, with no need for repeat treatment [28]. In addition, in 2011, Saadeldin [29] studied the electrophysiological features of these gastroenteritis-associated seizures using EEG on 25 pediatric patients. Among the included patients, 32% were affected by focal, tonic or clonic seizures, 12% by focal with secondary generalization seizures, 36% by generalized tonic-clonic seizures and 20% consisted of staring only (absence-like) with no motor components. In this study, 52% of patients had clusters of seizures and 48% had isolated seizures. Interictal EEGs and brain imaging were normal for all the patients. No patients required treatment with antiepileptic drugs. All the patients were found to have normal psychomotor development and neurological examination after a follow-up period between 15 and 56 months. Nevertheless, the authors noted the risk of bias in their study due to the small sample size and that similar studies with a larger population were needed. In addition, similar neurological manifestations were described by Lee and Ong [30] as a distinct type of situation-related seizures, which they named "afebrile seizures associated with minor infections". Furthermore, Zerr *et al.* [31] reported 36 children with first-time

afebrile seizures occurring in the context of mild illness, characterized mainly by diarrhea, vomiting, and rhinorrhea, which they named “non-febrile illness seizures”. The authors compared the electro-clinical features of these children with those of children affected by febrile convulsions and unprovoked seizures [31]. Their data demonstrated an association between peripheral inflammation and ‘non-febrile illness seizures’, and this condition appeared to have occurred as episodes of unprovoked seizures, showing common features with febrile seizures. Therefore, the results of these studies show an immunological link between peripheral inflammation of various causes and CNS inflammation, although further case–control studies are needed to clarify the exact nature of this link.

Typical clinical presentation of CMA

The typical presentation of CMA usually involves the gastrointestinal tract, manifesting with frequent regurgitations, gastroesophageal reflux, vomiting, diarrhea and/or constipation, abdominal pain and abdominal colic. Literature data suggest that CMA clinical signs and symptoms predominantly appear associated with gastrointestinal symptoms with a range of 32–60% of cases, while cutaneous and respiratory symptoms manifest in 20–40% of cases, and anaphylaxis can occur in 0.8 to 9% of cases [32–39]. Nevertheless, few studies have shown neurological involvement of CMA.

Epilepsy & CMA

Hypotheses on the relationship between epilepsy and atopic diseases were first postulated by Spangler, University of Philadelphia, who suggested that idiopathic epilepsy seemed to be linked to ill-defined experimental, hereditary, biochemical, clinical and therapeutic factors, running in some way parallel to allergy and essential (nonorganic) epilepsy. According to Spangler, periodic convulsive phenotypes may represent a response of the immune system to an allergic metabolic disturbance [40]. Later, in 1981, Gray published in the British Medical Journal his considerations on a specific addressed question from a doctor who had met 10 children with epilepsy in his practice, all of whom had at least one first-degree relative who had a history of reacting to some allergens [41], suggesting that atopic manifestations develop in individuals, particularly children, who have a disordered ‘immunological vocabulary’, cross-reacting with the neural immune system in the pathogenesis of epileptic seizures.

Since then, there have been many research attempts to demonstrate a possible relationship between the two diseases [42–46]. In 1998, Castaneda *et al.* [43] led a retrospective study on 400 asthmatic patients, regularly followed-up in the “Asthma and Allergy Clinic of the New York University Medical Centre” and 201 consecutive patients with idiopathic epilepsy followed at the Pediatric Neurology Clinic of the same hospital. They found a prevalence of 0.75% for idiopathic epilepsy among the 400 cases of asthma and the prevalence of epilepsy was similar in both mild (0.79%) and moderate-to-severe (0.73%) asthma. They further found a 5.97% prevalence (12 cases) of asthmatic children among the

201 cases of idiopathic epilepsy. Nevertheless, they found that prevalence rates for both diseases were similar to those found in the general population. Therefore, the authors concluded that idiopathic epilepsy and asthma are not etiologically related or mutually predisposing conditions. One year later, the Italian research group of La Sapienza University of Rome reported three pediatric cases of cryptogenetic partial epilepsy, diagnosed by EEG and associated with behavioral disorders (sleep disorders, hyperactivity and writing difficulties), whose symptoms notably improved after a cow’s milk-free diet. Their clinical improvement reflected an improvement of behavioral symptoms, followed by normalization of EEG anomalies. Upon double blind oral provocation tests, these patients did not display any immediate reaction, but after a few days resuming on a controlled diet, normalization of EEG results was observed in all cases [44]. The same Italian research group [45], in 2002, led a retrospective study on 72 epileptic children and 202 controls, who were investigated for the presence of allergy together with their immediate families. They found a statistically significant higher rate of eczema in epileptic patients’ mothers and rhinitis in their siblings with respect to controls. Later, in 2012, the same research group postulated a relationship between cow’s milk allergy and Rolandic epilepsy [46]. The authors enrolled 56 children, aged 4 to 14 years, suffering from Rolandic epilepsy with high frequency of fits. They treated Rolandic epilepsy with anticonvulsant drugs in non-allergic patients, while for those with a clear diagnosis of atopy a cow’s milk-free diet was started. They found that children of the non-allergic group displayed complete clinical and EEG remission in 29 out of 39 cases (74%). The cow’s milk-free diet made it possible to obtain clinical remission in 17 out of 20 cases (80%) of the allergic group with EEG normalization in these patients. Moreover, the group treated with anticonvulsants had hypertransaminasemia and slight leucopenia, whereas no side effects were detected for the CM protein-free diet group [46]. Taken together, these studies suggest a higher prevalence of atopic disorders in children affected by seizures and vice versa (TABLE 1). These findings, therefore, emphasize the need of further studies that could explain the ‘trait-d’union motif’ between the two diseases, proposing a common immune pro-inflammatory pattern for both diseases.

Neuroinflammation & BBB

It has been demonstrated that immune dysfunction and inflammation alter the BBB integrity [47,48], and it seems that the loss of this integrity, especially in the leukocyte-endothelial adhesion ability, may also be involved in the pathogenesis of epilepsy (TABLE 2) [49]. This event was described by Ransohoff using the term ‘immunological barrier to electrical storms’ [50], from evidence that mast cells were considered the ‘immune gate to the brain’ [51], located in the BBB itself. These mast cells seem to be activated by any kind of stress, such as oxidative stress [52], and/or stress hormones release such as corticotropin-releasing hormone, that has been demonstrated to cause a BBB disruption. The possible involvement of mast cells is moreover supported by the property of histamine-1 receptors that increases the frequency of epileptic events [54].

The disruption of the BBB by the activation of brain mast cells leads to local neuronal inflammation that could constitute an epileptogenic focus. This process could worsen by activation of Fc- γ receptors (Fc γ RI) on neurons, contributing to brain cell death observed after injection of the pro-convulsant compound kainic acid [55–57]. Recently, another type of Fc receptor was identified in neurons, the Fc-epsilon receptors (Fc ϵ RI) [58,59]. These receptors were typically thought to be expressed only by mast cells and basophils however their identification on neurons seems to imply that allergic triggers may directly affect the neurons after BBB disruption, permitting a secondary entry of immunoglobulins.

The above-mentioned studies led to the production of reflective papers on clinical patients affected by signs of BBB disruption. Furthermore, other proteins have been implicated in neuroinflammation with recent publications showing that increased serum levels of 'high mobility group box 1 protein', released from neurons following neurotoxicity, in young autistic patients [59–61], activate a pro-seizure pathway via toll-like receptor 4 activation in mice [62]. Moreover, damaged associated molecular patterns, including mitochondrial DNA, were reported in trauma patients to be released from damaged cells and activate toll-like receptor with a consequent auto-inflammation [63]. Furthermore, mitochondrial DNA has been shown to be neurotoxic and alter behavior by directly damaging neuronal cells in mice [64].

The concept of the presence of neuroinflammation opens the possibility of treating seizures associated with immune-mediated diseases, such as ASDs and mastocytosis, in which anticonvulsant drugs are often ineffective [65]. In these cases, treatment approaches directed to brain mast cells activation and inflammation may have a major effect, as well as the use of molecules with antioxidant and anti-inflammatory abilities, such as natural flavonoids [66].

Neuroinflammation & T lymphocytes subsets

In the pathogenesis of inflammation, the balance between lymphocytes secreting pro-inflammatory cytokines and those producing anti-inflammatory cytokines plays a key role in starting and perpetuating the inflammatory process. It may be possible to speculate that an imbalance of various pro-inflammatory and anti-inflammatory T lymphocytes could be responsible for processes of neuroinflammation involved in epileptogenesis.

Recently, in 2013, He *et al.* [67] have demonstrated in human samples that levels of IL-17, IL-17 receptor (IL-17R) and downstream factors of the IL-17 pathway (such as NF- κ B activator 1), all cytokines with a pro-inflammatory activity, were markedly elevated in focal cortical dysplasia type Ia, IIa and IIb. Moreover, IL-17 and IL-17R levels directly correlated with the increased frequency of seizures in these patients. The authors found, by immunostaining, that IL-17 and IL-17R were highly expressed in dysmorphic neurons, neuronal microcolumns, astrocytes, balloon cells and vascular endothelial cells, while NF- κ B activator 1 and NF κ B-p65 were widely expressed in patients with focal cortical dysplasia. Thus, the authors concluded that IL-17 system overexpression and IL-17 signal transduction pathway activation may be involved in the epileptogenic process of cortical lesions in focal cortical dysplasia.

Janigro [68] published a review on permeability across the epileptic barrier, challenging the theory that epileptic seizures can only be initiated by a foci of 'epileptic' neurons. After the recognition of astrocytic-neuronal communication, and the close crosstalk between astrocytes and brain endothelial cells, it has been supposed that the BBB acts as a 'neurovascular unit'. These hypotheses led to new frontiers in epileptogenic research, focusing on investigations of cerebral blood flow, permeability of cerebral microvessels and leukocyte adhesion molecules at the BBB that have been proposed initiate pilocarpine-induced status epilepticus. Furthermore, BBB disruption itself was demonstrated to be a trigger factor for seizures, with anticonvulsant effects obtained by administration of strong anti-inflammatory 'BBB repair' drugs [69].

It is not known how systemic inflammation becomes active before an ictal event. It seems that pilocarpine, and other receptor-specific convulsive agents such as muscarin molecules, can activate the innate immune system by acting on T and B cells [69–73]. Furthermore, pilocarpine acts on leukocytes expressing muscarinic, cholinergic and nicotinic receptors and that muscarinic receptors are fundamental for CD8⁺ cytotoxic T-lymphocyte expression, as seen in experimental mice models with targeted deletions of each of the known muscarinic receptors (M1–M5). These studies have demonstrated that CD8⁺ T cells from M1 receptor-deficient mice were defective in differentiating into cytotoxic T lymphocytes [73]. These findings are therefore consistent with results linking pilocarpine-induced seizures to CD8⁺ T cell activation and mobilization from the spleen [73,74]. Pilocarpine, when injected systemically, activates T cells to acquire a cytotoxic (CD8⁺) phenotype in the spleen; these events lead to an inflammatory response that via unknown pathogenic pathways causes BBB leakage in highly epileptogenic areas, such as the limbic system. It seems that in addition to T cells, other cell types are involved. It has also been demonstrated that pilocarpine-induced seizures, consistent with pilocarpine inflammatory action, are prevented by IL-1b antagonists or steroids [71–76]. Furthermore, Costa-Ferro *et al.* have recently found that transplantation of mononuclear bone marrow cells in murine models affected by pilocarpine-induced epilepsy effectively decreased seizure frequency and duration and levels of pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6. Moreover, transplants performed at seizure onset protected against pilocarpine-induced neuronal loss and gliosis and stimulated the proliferation of new neurons in epileptic rats [74].

In addition, the involvement of T-lymphocytes in epileptogenesis has also been demonstrated by Deprez *et al.* [77], who investigated the role of adaptive immunity epileptic animal models by intravenous transfer of immunopurified T cells in mice lacking CD8⁺ T cells (β 2-microglobulin-knockout), CD4⁺ T cells (MHCII-knockout), or both (RAG1-knockout mice). It has been demonstrated that KA, when injected in intra-hippocampal areas of adult mice, can cause a focal lesion in the CA1 area and hilus of the dentate gyrus and marked granule cell hypertrophy and dispersion leading to the onset of chronic focal seizures. These critical events are preceded by infiltration of T lymphocytes into the damaged tissue and macrophage-like cells into the dentate gyrus.

The authors found that depletion of CD4⁺ and/or CD8⁺ T lymphocytes by targeted gene deletion results in decreased latency to seizure onset, consequently suggesting a key role for adaptive immunity in seizure onset. Furthermore, Deprez *et al.* [77] demonstrated that EEG analysis in mutant mice in which kainic acid was injected showed that grafting of the missing T cell population, 2 days after the T cell transfer, had no influence on epileptogenesis, but strongly influenced F4/80⁺ macrophage-like cell infiltration in the dentate gyrus. Moreover, the authors found that macrophage recruitment was increased by CD8⁺ T cells in β 2-microglobulin-knockout mice, while CD4⁺ T cells transferred in MHCII-knockout and in RAG1-knockout mice blocked macrophage infiltration, thus causing decreased granule cell dispersion and survival and worsening the kainic acid-induced lesion. They concluded that intact adaptive immunity, T-cells and mononuclear phagocyte complex cross-talk have a key role in the control of neuronal integrity and survival in the damaged brain [77,78].

However, Offner *et al.* demonstrated that central damage can also affect the systemic immune response as well as peripheral inflammation which in turn could affect the neurons [79]. They showed that induction of stroke not only produces local ischemia and brain damage but also has profound effects on peripheral immune responses. They evaluated the effects on spleen and blood cells 4 days after stroke induction. They found a decrease in inflammatory cytokines in the middle cerebral artery occlusion-affected right brain hemisphere 96 h after stroke onset compared with earlier time points [79]. They further demonstrated that stroke leads to splenic atrophy with a decrease of the organ size, a drastic loss of splenocytes and induction of annexin V⁺ and TUNEL⁺ cells within the spleen, with consequent reduction of T-cell proliferation responses and secretion of inflammatory cytokines. These changes produced a state of profound immune suppression, characterized by a drastic reduction in B-cell numbers in spleen and blood, and a novel increase in CD4⁺FoxP3⁺ regulatory T-cells [79]. The authors concluded that their results thus provided evidence to support the hypothesis that CNS damage caused by cerebral ischemia was able to cause a drastic state of immunosuppression, characterized by cell death as well as an increased presence of peripheral CD4⁺FoxP3⁺ regulatory T cells [79].

Taken together the above studies confirm that a state of neuronal inflammation could be the basic trigger for epileptic seizures; however, it is still unknown how a systemic immune imbalance could affect neuronal homeostasis and what kind of patients undergoes immune-mediated seizures. Moreover, the involvement of Th17 in the pathogenesis of 'inflammatory seizures' may raise the speculation that Treg cells are indirectly involved in this process, although further studies are needed to clarify their role in epileptogenesis.

Cow's milk allergy & convulsive episodes: the description of our pediatric case

S.J., a 40-day-old child, was admitted to our Pediatric Operative Unit, Vittorio Emanuele Hospital, University of Catania, Italy. Because he was 10 days old he had experienced frequent

episodes of vomiting and diarrhea, associated with episodes characterized by generalized clonic movements, with loss of consciousness, lasting about 5–10 min with spontaneous recovery. He was born at term (after 38 weeks gestation), small for gestational age (weight: 1800 g and height 48 cm), by caesarean section, of a twin pregnancy. However, his twin died in utero during the 8th week of gestation.

At birth, the child was hospitalized in the NICU Department of a nearby hospital, for intra-uterine growth failure.

His physical examination revealed that his auxologic parameters were slightly under the normal range (weight: 3100 g [$<3^{\circ}$ centile], height: 49 cm [3° centile], head circumference: 36 cm [3 – 10° centile]). His neurologic exam showed the presence of generalized muscle hypertonia, alternating with phases of generalized hypotonia and marked rotula hyperreflexia.

At admission the child underwent an arterial blood gas analysis, which showed the presence of respiratory acidosis (pH: 7.31, PCO₂: 38 mm Hg, PO₂: 51 mm Hg, HCO₃⁻: 19.2 mmol/l), with a transcutaneous O₂ saturation of 100%. During the hospitalization, he underwent serial measurements by blood gas analysis of his metabolic parameters, such as electrolytes, lactic acid and bicarbonate that were always within the normal range.

Routine blood tests (hematinics with white cell count, urea and electrolytes, renal and kidney function tests, immunoglobulins, hematinics, stool and urine culture, Fehling test, blood ammonium and lactate and tandem mass test) were all in the normal range, with the exceptional presence of a slight anemia (Hb: 10.3 g/dl). We therefore excluded the principal metabolic diseases associated with aminoacidemia, urinary organic acid, galactosemia and tandem mass.

During hospitalization, the child had recurrent episodes of vomiting after cow's milk consumption, often associated with leg tremors, episodes of hypertonia followed by hypotonic, retropulsion of his ocular globes and clonic movements of his inferior lip. Video EEG showed a burst suppression-like pattern with paroxysmal elements in bilateral temporal regions.

The child underwent further diagnostic exams, including ophthalmic specialist review, allergy specialist review, skin prick test for cow's milk protein, ECG, cardiac ultrasound scan, cerebral MRI that were all normal, with the exception of his chest X-ray that showed the presence of: 'a median large shadow of timic nature and marked broncho-vascular trauma'.

His abdomen ultrasound scan showed the presence of 'slight congenital left hydronephrosis'.

Three days after his admission, we repeated a venous blood gas analysis that showed only little improvement (pH: 7.32, PCO₂: 54 mm Hg, PO₂: 22 mm Hg, HCO₃⁻: 23.2 mmol/l).

During the 10th day of hospitalization, the child's gastrointestinal symptoms persisted with the same frequency and intensity, and his neurological condition was unchanged, as far as his EEG was concerned. Therefore, as these episodes were often associated with the consumption of cow's milk, the child started an amino acid infant formula, lacking cow's milk protein (Neocate, Nestlé). After beginning this diet, his clinical condition abruptly improved, with resolution of his leg tremors

and the episodes of alternating hypertonia and hypotonia. His blood gas analysis parameters also improved (pH: 7.36, PCO₂: 39 mm Hg, PO₂: 51 mm Hg, HCO₃⁻: 23.6 mmol/l), as well as his video EEG exam which was consistent with his chronologic age, with resolution of his pattern of neurological impairment.

One month after the onset of the amino acid infant formula, the child underwent a cow's milk protein challenge test, using an infant formula with cow's milk protein, but without galactose, to differentiate from any form of alteration in galactose metabolism. The challenge was performed according to the standard international protocols [1], and was positive, with episodes of vomiting and diarrhea and the onset of suction movements when the child reached the dose of 150 mg of cow's milk. Moreover, during the challenge a video EEG was performed, and when the child consumed doses of between 35 and 70 mg of cow's milk, the exams showed a generalized slowing of his basal activity.

Due to the positive challenge test and the improvement of clinical, laboratory and EEG parameters after the cow's milk protein-free diet, a diagnosis of CMA was performed and the amino acid infant formula was continued.

At follow-up visit, the child was in good health, his auxologic parameters reached the 25–50^o percentile, and his parents did not report further episodes of vomiting and/or diarrhea or neurological symptoms.

We described the above case for the peculiar clinical association between gastrointestinal symptoms in CMA, and neurological symptoms, presenting as seizures and alteration of muscle tone. As explained above, a possible common immune pattern could be the cause of this unusual association, perhaps due to some kind of immune disruption of the child's BBB. A figurative explanation of this hypothesis is shown in FIGURE 1. An important consideration rising from this case is that the child was born from a twin pregnancy, in which the brother died at 8 weeks of gestation. This event may have caused an immune dysregulation towards the enhancement of pro-inflammatory processes and the production of oxidative stress that lead to IUGR. Furthermore, a systemic pro-inflammatory immune response has been associated with a BBB disruption and this event would explain the association between the consumption of cow's milk and seizures after the onset of gastrointestinal symptoms. However, as there are no studies on the

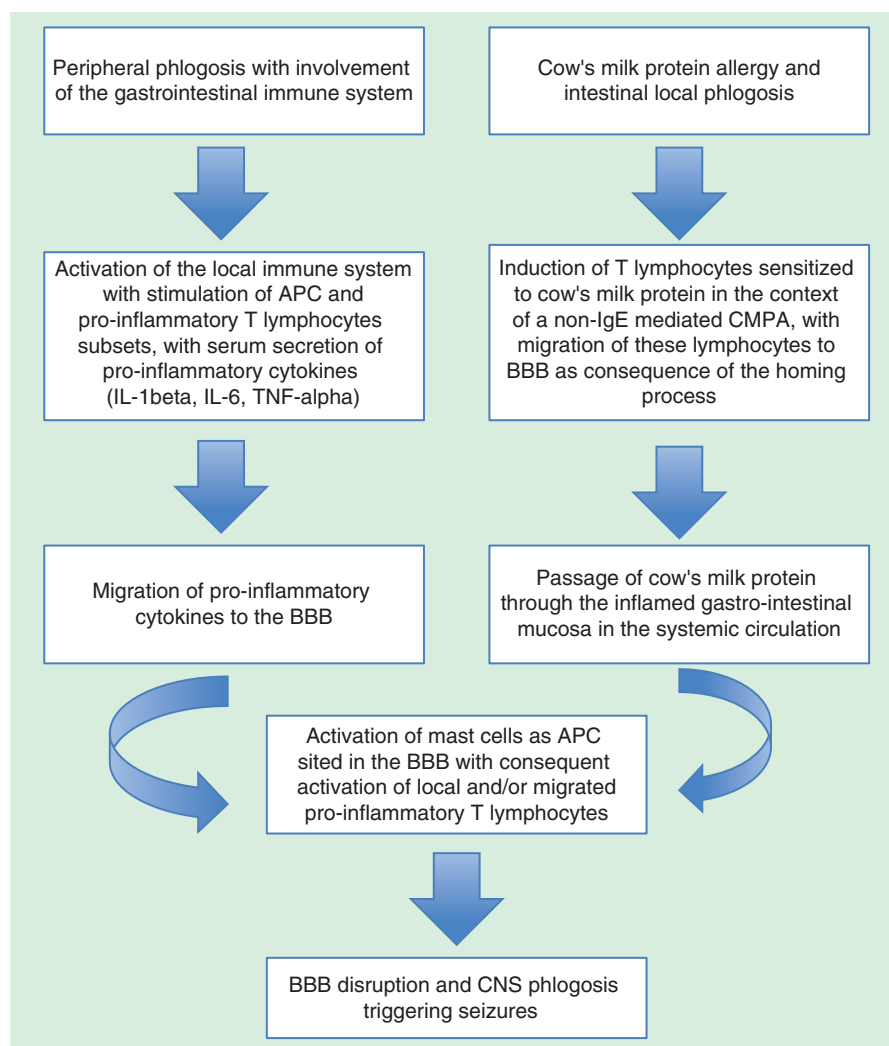


Figure 1. Diagram on the pathogenic hypothesis of the link between peripheral phlogosis induced by cow's milk protein allergy and CNS phlogosis triggering seizures.

APC: Antigenpresentingcells; BBB: Blood–brain barrier. CMPA: Cow's milk protein allergy.

fetal immune response after a twin death, further studies are needed to clarify the pathogenic events leading to neuroinflammation in this unusual pathological situation. It has been recently shown that intrauterine fetal death of one twin, of diamniotic twins, is associated with adverse perinatal outcome (lower Apgar score, lower birth weight, higher rates of preterm birth before 34 weeks of gestation) [80]. Furthermore, in case of fetal death in monochorionic twins, a higher rate of neurodevelopmental morbidity has been subsequently described [81].

This case could be considered a precursor leading to new frontiers of research on neuroinflammation in atopic diseases.

Conclusion

Recent literature, experimental and clinical data are trying to explain the multisystem inflammatory state caused by cow's milk proteins, giving a reasonable explanation of extraintestinal phenotypic manifestations of CMA. In light of the established

neurological manifestations of CMA, we suggest that the complex immunological mechanisms that regulate the allergic reaction may play a key role in these epileptic manifestations in the context of CMA. Most current studies refer to IgE-mediated allergic reactions linked to epileptic seizures, whereas few data are available on the link between non-IgE-mediated reactions and neurological manifestations. Thus, in the causal diagnosis of epileptic seizures, when an allergic reaction is suspected, we suggest further investigation of both IgE- and non-IgE-mediated reactions, considering this topic as a new frontier of research on neuroinflammation in atopic diseases.

Expert commentary

Our review focuses on the neurological manifestations of CMA. The importance of our review is attributable to the diagnosis of this unusual presentation of CMA and a possible etiological explanation, involving immune-mediated CNS damage that, through BBB disruption operated by pro-inflammatory cytokines, leads to neurological manifestations. It is not still clear if the immune component in the CNS is a response to peripheral inflammation or if these pro-inflammatory cytokines are locally produced. Nonetheless, we report evidence that CNS inflammation triggers seizures occurring in CMA. We believe that our

review is innovative to highlight inflammation as a possible target for anticonvulsive treatment.

Five-year view

The importance of targeting inflammation as a trigger for seizures relies on the possibility of optimizing anticonvulsive therapy, driving research strategies towards anti-inflammatory immunotherapies. The actual research efforts are focusing on immunomodulatory therapies to decrease the inflammation in drug-resistant epilepsies, showing an increased efficacy in the treatment of these pathological entities, with less adverse reactions than anticonvulsive treatment itself. Therefore, the knowledge of the CNS inflammation in allergic diseases is fundamental to open new research topics for immunomodulating therapeutic strategies.

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Key issues

- The prevalence of food allergy, in the global population, accounts for 220–520 million affected people and represents a major global health burden.
- Usually, the gastrointestinal symptoms are recognized as a ‘typical’ presentation of the disease, with vomiting, diarrhea and/or constipation and gastroesophageal reflux. Nevertheless, recently ‘atypical’ symptoms, involving the CNS and the peripheral nervous system have been described. These symptoms can occur both in IgE- and non-IgE-mediated cow’s milk protein allergy, as expression of a multisystem spread of immunogenic mechanisms that occur due to cow’s milk protein allergy.
- After lymphoid follicles T and B cells activation, they migrate via the lymphatic and the circulation system to several target organs. This process is called ‘homing’. In a patient in whom tolerance is not achieved, T and B cells will be activated at a homing site by direct contact with a specific food antigen, releasing pro-inflammatory cytokines, antibodies and vasoactive peptides and causing an inflammatory reaction in the affected organ.
- It could be speculated that an increased intestinal permeability, secondary to an inflammation, can cause the systemic passage of cow’s milk allergens and by the ‘homing’ process perpetuate a state of inflammation in other sites distant from the gastrointestinal tract, such as the blood brain barrier. This mechanism would explain the unusual neurological manifestations, as recently described in the literature, in children affected by cow’s milk protein allergy.
- Hypotheses on a relationship between epilepsy and atopic diseases dates back to the first writings of Spangler RH, of the University of Philadelphia, who supposed that the mechanism ‘idiopathic epilepsy’ to be likely parallel in allergy and essential (nonorganic) epilepsy. These hypotheses are supported by papers that were published from this first assumption until recent research data.
- It has been demonstrated that immune dysfunction and inflammation appear to alter the blood–brain barrier integrity, and it seems that the loss of this integrity, especially leukocyte endothelial adhesion may also be somehow involved in epileptogenesis. This arises from evidence that mast cells were considered the ‘immune gate to the brain’.
- Taken together the studies contained in the review confirm that a state of neuronal inflammation could be the basic trigger for epileptic conditions, nevertheless it is still unknown how a systemic immune imbalance could affect neuronal homeostasis and what kind of patients are susceptible to immune-mediated seizures.
- In the causal diagnosis of epileptic seizures, when an allergic reaction is suspected, we suggest further investigation of the possibility of both IgE- and non-IgE-mediated reactions. This topic is a potential new frontier of research on neuroinflammation in atopic diseases.

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Perspective, ~~US English~~

Epileptic seizures as a manifestation of cow's milk allergy: ~~A~~^a studied relationship and description of our pediatric experience

Epileptic seizures as a manifestation of cow's milk allergy

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Adverse reactions after ingestion of cow's milk proteins can occur at any age, from birth and even amongst exclusively breast-fed infants, although not all of these are hypersensitivity reactions. The most common presentations related to cow's milk protein allergy (~~CMA~~) are skin reactions, failure to thrive, anaphylaxis as well as gastrointestinal and respiratory disorders. In addition, several cases of cow's milk protein allergy~~CMA~~ in the literature have documented neurological involvement, manifesting with convulsive seizures in children. This may be due to ~~central nervous system~~ (~~CNS~~ spread of a peripheral inflammatory response. Furthermore, there is evidence that pro-inflammatory cytokines are responsible for disrupting the blood-brain barrier, causing focal CNS inflammation thereby triggering seizures, although further studies are needed to clarify the pathogenic relationship between atopy and its neurological manifestations. This review aims to ~~analyse~~^{analyze} current published data on the link between cow's milk protein allergy~~CMA~~ and epileptic events,

highlighting scientific evidence for any potential pathogenic mechanism and describing our clinical experience in paediatrics.

Keywords: atypical clinical features • ~~central nervous system~~CNS inflammation • cow's milk allergy • pro-inflammatory cytokines • seizures

The global prevalence of food allergy is estimated to range between 220–~~and~~ 520 million people ¹. Adverse reactions after ingestion of cow's milk proteins can occur at any age from birth, and even among~~st~~ exclusively breast-fed infants, although not all of these are hypersensitivity reactions ¹.

In general, food allergy is more frequent in childhood than in adulthood; and according to a recent Japanese multicentre study, the prevalence of cow's milk protein allergy (CMA) is 0.21% in newborns and 0.35% among~~st~~ extremely premature babies (~~Birth-birth~~ weight <1000 g) ².

In a large European survey ³, in which more than 44,000 individuals were contacted, data of 8,000 children living in Austria, Belgium, Denmark, Finland, Germany, Greece, Italy, Poland, Slovenia and Switzerland was obtained. For each telephone contact, a multiple choice questionnaire to assess CMA epidemiology, clinical signs and symptoms and chosen treatment was submitted to each individual of the family. The estimated prevalence of CMA in this study was 4.7%, with the highest rate in children between 2 ~~and~~ 3 years of age (7.2%). Wide national differences were noted, with prevalence rates ranging from 13.8% in Greece to 52.3% in Finland. In addition, ~~Milk-milk~~ was the most frequently reported offending food in children (38.5% of atopic cases) and the second most frequently implicated in adulthood (26%). Furthermore, the most frequent allergic manifestations of CMA were: skin reactions (70% of cases), gastrointestinal (25%) and respiratory (20%) symptoms. In 70% of cases, a therapeutic treatment was needed. However, it is important to differentiate CMA from cow's milk protein intolerance (~~CMPH~~), which is a distinct entity with some similarities to CMA. This is present in the western world with an incidence around 2–5% among~~st~~ newborns ⁴. It is fundamental for paediatricians to differentiate between cow's milk protein intolerance~~CMPH~~ and

CMA ~~in order~~ to diagnose those forms of intolerance usually evolving towards CMA, exposing these patients to a high risk of anaphylaxis ⁵.

CMA typically presents with gastrointestinal symptoms, such as vomiting, diarrhoea and/or constipation as well as gastroesophageal-reflux. However, "atypical" symptoms, involving the Central (CNS) and Peripheral (PNS) Nervous Systems, have been recently identified. These symptoms can occur in both IgE- and non-IgE-mediated CMA, as an extension of the diffused, multi-organ inflammatory response to CMA. Furthermore, recent research attempts to explain the association between CMA and seizures have been led by Riazi *et al.* and colleagues, who showed a possible role for systemic inflammation in increasing neuronal excitability and leading to greater seizure susceptibility ⁶.

The aim of our review is therefore to ~~analyse~~ ^{analyze} the current literature on the link between CMA and epileptic events, highlighting the evidence that could explain a pathogenic link between the two diseases in conjunction with describing our experience in paediatrics.

Methods

Articles reporting evidence on the association between food allergy, particularly CMA, and neurological disease, with or without statistical meta-analysis, were selected. Those reporting evidence of one or more other non-allergic (comorbid) disorders were considered (outcome). Studies that assessed the relationship between atopic diseases with non-specific symptoms, such as psychosocial distress or sleeping problems, were excluded.

Given the lack of an electronic database that contains all publications of all medical journals and that a restriction of only one database could be associated with a systematic bias, it was necessary to combine multiple databases for a comprehensive literature search. For this reason, an electronic literature search was carried out in (1)-MEDLINE via PubMed interface, (2)-SCOPUS, (3)-Google

Scholar, ~~(4)~~ the Cochrane Library for all articles published from inception to October 2013.

~~Database-Database~~-specific search strings were developed and included search terms describing food allergy and/or CMA (population/exposure) and ~~Neurological-neurological~~ signs or symptoms (study design/description of cases). A combination of medical subject headings (~~MeSH~~) and keywords was used.

Titles and abstracts of identified papers were screened by two independent reviewers to determine whether they met the eligibility criteria of interest to develop our review. Subsequently, full texts of the remaining articles were independently retrieved by the two reviewers for eligibility.

Peripheral inflammation as trigger of seizures: ~~A~~ link between the gastrointestinal ~~and~~ & the neurological system

It has ~~been~~ recently ~~been~~ demonstrated that peripheral inflammation reflects a similar inflammatory state in the brain ⁶. The ~~Central Nervous System~~CNS inflammation is characterized by microglial activation with an increase of pro-inflammatory cytokines, such as ~~tumor necrosis factor-alfa~~-(TNF-~~alfa~~), IL-1~~beta~~ and IL-6 ⁶. Recent attempts have been made to study the link between peripheral and central inflammation, with evidence that cytokines can directly influence *in vitro* synaptic functions and neural properties ^{7,8,9,10,11,12} or can alter transmitter biosynthetic pathways ^{13,14}. Nevertheless, the pathogenic mechanism that links peripheral and central inflammation is still poorly understood, despite inflammation being recognized as an important trigger for seizures, it is pertinent to investigate its potential role in mediating this pathogenic relationship. However, despite the link between CNS inflammation and seizures being well established, poor data are available on the link between peripheral inflammation and seizures as well as knowledge of the relationship between peripheral inflammation and neuronal excitability (~~Tables 1 & 2~~) ^{15,16}.

~~Since-Because~~ the hippocampus is fundamental for the onset and propagation of seizures, Riazi *et al.* [6] have shown how peripheral inflammation can alter hippocampal neuronal excitability studying a murine model of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. [2,4,6]

~~Trinitrobenzene-sulfonic acid~~ ~~TNBS~~ ~~TNBS~~ is a Th1-cell-mediated model of inflammatory bowel disease (~~IBD~~) that during acute phases is associated with activation of the humoral immune system, causing a localized inflammatory colitis [17,18,19]. Furthermore, Riazi *et al.* identified a model of microglial-dependent, TNF-mediated increase in CNS excitability that may be considered a potential link between peripheral and central inflammation. The clinical evidence of this relationship was demonstrated by the same authors through a reversible increase in seizure susceptibility, directly correlating with the grade of severity of inflammation and hippocampal electrophysiological recordings of inflamed mice. These findings revealed increased excitability and epileptiform burst discharges to bath application of potassium channel blocker [r1] 4-AP, as expressed by increased excitability of neuronal networks in the hippocampus following peripheral inflammation. These findings, therefore, suggest that the production of TNF-~~alpha~~ occurs within the brain during peripheral inflammation, increasing seizure susceptibility. Moreover, increased TNF-~~signallings~~ ~~signaling~~ in the CNS as well as microglial activation, appeared sufficient for changes in seizure susceptibility. Furthermore, the authors also noted that colonic inflammation lowered the threshold for the induction of IV ~~Pentylentetrazole~~ ~~pentylentetrazole~~ (PTZ)-induced seizures. A possible pathogenic mechanism was suggested which alters ~~pentylentetrazole~~ ~~PTZ~~ ~~Pentylentetrazole~~ metabolism due to a disruption of the ~~blood-blood~~ brain barrier (BBB) that occurred in this inflammatory model [20], eventually resulting in an increased uptake of ~~pentylentetrazole~~ ~~PTZ~~ within the brain. Therefore, not only can this model potentially provide a better understanding of peripheral inflammation but also how this may present with features that involve the CNS, giving new insights into co-morbidities resulting from altered CNS function in the context of peripheral inflammatory diseases [6].

The immune mechanism of epileptogenesis as a consequence of peripheral inflammation has centered upon the role of microglia. Microglial activation as a consequence of peripheral inflammation was confirmed by the increased number of microglial cells in the hippocampus and adjacent areas [21,22,23]. In keeping with this observation, the authors showed an increased production of hippocampal TNF- α , despite low IL-1 levels, explaining that these findings were likely related to the colonic inflammatory model, which at the height of colic inflammation is associated with a downregulation of IL-1 synthesis in comparison with TNF- α secretion. These findings have been supported by literature implicating pro-inflammatory cytokines in seizure activity and epilepsy [24,25,26,27], with a predominant role for IL-1 β as a pro-convulsing cytokine and IL-1 receptor antagonist (IL-1Ra), mainly playing an anticonvulsant role. However, Riazi *et al.* show in their studies that IL-1Ra did not have any significant effect on epileptogenesis whereas the blocking of central TNF- α was alone effective as an anti-convulsant mechanism. The authors therefore had good evidence to conclude that TNF- α was responsible for microglial activation and consequently triggering seizures. According to this evidence, it might be expected that chronic exposure to CNS TNF- α might induce a decrease in seizure threshold. On the contrary, Riazi *et al.* [6] found that a 4-day TNF- α infusion into the lateral ventricle of the mouse models (a time sufficient to induce maximum colitis and seizure susceptibility) caused a significant decrease in seizure threshold. The magnitude of the seizure susceptibility change was slightly weaker than what was observed after 2,4,6-trinitrobenzene sulfonic acid TNBS-induced inflammation. This phenomenon could be related to the incomplete diffusion of exogenous TNF- α to all brain areas, such as the amygdala and the endorhinal cortex, both distant from the ventricles and involved in seizure generation.

This link between peripheral and CNS inflammation, and in particular gastrointestinal inflammation and seizures, through the involvement of TNF- α and IL-1 β cytokines, may explain the neurological involvement in clinical gastroenteritis recently described in the literature. Riazi *et al.*

showed this basic link using experimental evidence whereas other researchers have led studies in the clinical field to support this hypothesis. Recently, Maseri ~~et al.~~ (2013) ²⁸ studied the incidence of gastroenteritis-related seizures. The authors prospectively collected data on all cases of paediatric seizures associated with gastroenteritis without electrolyte abnormalities, over a period of 2 years. They then divided the included patients in two groups, according to the presence or absence of fever during the convulsive episode. The authors studied 14 cases, from a reference population of 39,000 patients, characterized by a homogeneous phenomenological presentation of the convulsive episodes. They found that the onset of convulsive episodes was earlier in those patients affected by febrile gastroenteritis and that in 13/14 cases the ictal phenomenology was consistent with generalized convulsive episodes of which 7/7 were tonic-clonic seizures, 5/5 generalized hypertonia and one generalized hypotonia. Only in one case, the convulsive episode presented without fever, manifesting as a partial seizure with oculo-facial deviation towards the left side, along with clonus of the patient's upper lip. The authors estimated an incidence of gastroenteritis-related-seizures of 1/10,000 children for afebrile seizures associated with gastroenteritis, with an earlier onset of symptoms with respect to those patients affected by febrile seizures. Moreover, in the studied patients, an EEG was performed and in most cases a normal baseline activity was found, while non-specific and transitory abnormalities were found in 5/5 cases. The seizure episodes presented with recurrent features in 9/14 patients, while in 7/7 patients these episodes lasted up to 4 minutes and only in one patient more than 20 minutes. Fifty per-cent of patients were treated with rectal Diazepam, with no need for repeat treatment ²⁸. In addition, ~~In~~ ⁱⁿ 2011, Saadeldin ~~et al.~~ ²⁹ studied the electrophysiological features of these gastroenteritis-associated seizures using EEG on 25 paediatric patients. Among the included patients, 32% were affected by focal, tonic or clonic seizures, 12% by focal with secondary generalisation-generalization seizures, 36% by generalised-generalized tonic-clonic seizures and 20% consisted of staring only (absence-like) with no motor components. In this study, 52% of patients had clusters of seizures and 48% had isolated seizures. Interictal EEGs and brain imaging were normal for all

the patients. No patients required treatment with antiepileptic drugs. All the patients were found to have normal psychomotor development and neurological examination after a follow-up period between 15 and 56 months. Nevertheless, the authors noted the risk of bias in their study due to the small sample size and that similar studies with a larger population were needed. In addition, similar neurological manifestations were described by Lee and Ong [30] as a distinct type of situation-related seizures, which they named “afebrile seizures associated with minor infections”. Furthermore, Zerr *et al.* [31] reported 36 children with first-time afebrile seizures occurring in the context of mild illness, characterized mainly by diarrhoea, vomiting, and rhinorrhoea, which they named “non-febrile illness seizures”. The authors compared the electro-clinical features of these children with those of children affected by febrile convulsions and unprovoked seizures [31]. Their data demonstrated an association between peripheral inflammation and “non-febrile illness seizures”, and this condition appeared to have occurred as episodes of unprovoked seizures, showing common features with febrile seizures. Therefore, the results of these studies show an immunological link between peripheral inflammation of various causes and CNS inflammation, although further case-control studies are needed to clarify the exact nature of this link.

Typical clinical presentation of CMA

The typical presentation of CMA usually involves the gastrointestinal tract, manifesting with frequent regurgitations, gastroesophageal reflux, vomiting, diarrhoea and/or constipation, abdominal pain and abdominal colic. Literature data suggest that CMA clinical signs and symptoms predominantly appear associated with gastrointestinal symptoms with a range of 32–60% of cases, while cutaneous and respiratory symptoms manifest in 20–40% of cases, and anaphylaxis can occur in 0.8 to 9% of cases [32, 33, 34, 35, 36, 37, 38, 39]. Nevertheless, few studies have shown neurological involvement of CMA.

Epilepsy and CMA

Hypotheses on the relationship between epilepsy and atopic diseases were first postulated by Spangler³⁹, University of Philadelphia, who suggested that idiopathic epilepsy seemed to be linked to ill-defined experimental, hereditary, biochemical, clinical, and therapeutic factors, running in some way parallel to allergy and essential (nonorganic) epilepsy. According to Spangler³⁹, periodic convulsive phenotypes may represent a response of the immune system to an allergic metabolic disturbance⁴⁰. Later, in 1981, Gray⁴¹ published in the British Medical Journal his considerations on a specific addressed question from a doctor who had met 10 children with epilepsy in his practice, all of whom had at least one first-degree relative who had a history of reacting to some allergens⁴¹, suggesting that atopic manifestations develop in individuals, particularly children, who have a disordered "immunological vocabulary", cross-reacting with the neural immune system in the pathogenesis of epileptic seizures.

Since then, there have been many research attempts to demonstrate a possible relationship between the two diseases^{42, 43, 44, 45, 46}. In 1998, Castaneda-Gay⁴³ *et al.*⁴³ led a retrospective study on 400 asthmatic patients, regularly followed-up in the "Asthma and Allergy Clinic of the New York University Medical Centre" and 201 consecutive patients with idiopathic epilepsy followed at the Paediatric Neurology Clinic of the same hospital. They found a prevalence of 0.75% for idiopathic epilepsy among the 400 cases of asthma and the prevalence of epilepsy was similar in both mild (0.79%) and moderate-to-severe (0.73%) asthma. They further found a 5.97% prevalence (12 cases) of asthmatic children among the 201 cases of idiopathic epilepsy. Nevertheless, they found that prevalence rates for both diseases were similar to those found in the general population. Therefore, the authors concluded that idiopathic epilepsy and asthma are not etiologically related or mutually predisposing conditions. One year later, the Italian research group of La Sapienza University of Rome reported three pediatric cases of cryptogenetic partial epilepsy, diagnosed by EEG and associated with behavioural disorders (sleep disorders, hyperactivity and writing difficulties), whose symptoms notably improved after a cow's milk-free diet. Their clinical improvement reflected an

improvement of behavioral symptoms, followed by normalization of EEG anomalies. Upon double blind oral provocation tests, these patients did not display any immediate reaction, but after a few days resuming on a controlled diet, ~~normalisation-normalization~~ of EEG results was observed in all cases [44]. The same Italian research group [45], in 2002, led a retrospective study on 72 epileptic children and 202 controls, who were investigated for the presence of allergy together with their immediate families. They found a statistically significant higher rate of eczema in epileptic patients' mothers and rhinitis in their siblings with respect to controls. Later, in 2012, the same research group postulated a relationship between cow's milk allergy and Rolandic epilepsy [46]. The authors enrolled 56 children, aged 4 to 14 years, suffering from Rolandic epilepsy with high frequency of fits. They treated Rolandic epilepsy with anticonvulsant drugs in non-allergic patients, while for those with a clear diagnosis of atopy a cow's milk-free diet was started. They found that children of the non-allergic group displayed complete clinical and EEG remission in 29/39 cases (74%). The cow's ~~milk-milk~~-free diet made it possible to obtain clinical remission in 17/20 cases (80%) of the allergic group with EEG normalization in these patients. Moreover, the group treated with anticonvulsants had hypertransaminasemia and slight leucopenia, whereas no side effects were detected for the CM protein-free diet group [46]. Taken together, these studies suggest a higher prevalence of atopic disorders in children affected by seizures and vice versa (Table 1). These findings, therefore, emphasize the need of further studies that could explain the "trait-d'union motif" between the two diseases, proposing a common immune pro-inflammatory pattern for both diseases.

Neuro-inflammation & ~~and BBB~~ blood-brain barrier

It has been demonstrated that immune dysfunction and inflammation alter the ~~BBB~~ blood-brain barrier integrity [47,48], and it seems that the loss of this integrity, especially in the leukocyte-endothelial adhesion ability, may also be involved in the pathogenesis of epilepsy (Table 2) [49]. This event was described by Ransohoff ~~RM~~ using the term "immunological barrier to electrical

storms” [50], from evidence that mast cells were considered the “immune gate to the brain” [51], located in the BBB itself. These mast cells seem to be activated by any kind of stress, such as oxidative stress [52], and/or stress hormones release such as corticotropin-releasing hormone (CRH), that has been demonstrated to cause a blood-brain barrierBBB disruption. The possible involvement of mast cells is moreover supported by the property of histamine-1 receptors that increases the frequency of epileptic events [54].

The disruption of the BBB by the activation of brain mast cells leads to local neuronal inflammation that could constitute an epileptogenic focus. This process could worsen by activation of Fc-gamma receptors (FcγRI) on neurons, contributing to brain cell death observed after injection of the pro-convulsant compound kainic acid (KA) [55,56,57]. Recently, another type of Fc receptor was identified in neurons, the Fc-epsilon receptors (FcεRI) [58,59]. These receptors were typically thought to be expressed only by mast cells and basophils however their identification on neurons seems to imply that allergic triggers may directly affect the neurons after BBB disruption, permitting a secondary entry of immunoglobulins.

The above-mentioned studies led to the production of reflective papers on clinical patients affected by signs of BBB disruption. Furthermore, other proteins have been implicated in neuroinflammation with recent publications showing that increased serum levels of “high mobility group box 1 protein- (HMGB1)”, released from neurons following neurotoxicity, in young autistic patients [59,60,61], activate a pro-seizure pathway via toll-like receptor 4 (TLR-4) activation in mice [62]. Moreover, Damaged-damaged Associated-associated Molecular-molecular Ppatterns (DAMPs), including mitochondrial DNA, were reported in trauma patients to be released from damaged cells and activate toll-like receptorTLR with a consequent auto-inflammation [63]. Furthermore, mitochondrial DNA has been shown to be neurotoxic and alter behavior by directly damaging neuronal cells in mice [64].

The concept of the presence of neuroinflammation opens the possibility of treating seizures associated with immune-mediated diseases, such as ASDs^[A2] and mastocytosis, in which anticonvulsant drugs are often ineffective^[65]. In these cases, treatment approaches directed to brain mast cells activation and inflammation may have a major effect, as well as the use of molecules with anti-oxidant and anti-inflammatory abilities, such as natural flavonoids^[66].

Neuroinflammation ~~and~~ T lymphocytes subsets

In the pathogenesis of inflammation, the balance between lymphocytes secreting pro-inflammatory cytokines and those producing anti-inflammatory cytokines plays a key role in starting and perpetuating the inflammatory process. It may be possible to speculate that an imbalance of various pro-inflammatory and anti-inflammatory T lymphocytes could be responsible for processes of neuroinflammation involved in epileptogenesis.

Recently, in 2013, He^[67] *et al.* have demonstrated in human samples that levels of IL-17, IL-17 receptor (IL-17R) and downstream factors of the IL-17 pathway (such as nuclear factor- κ B activator 1), all cytokines with a pro-inflammatory activity, were markedly elevated in focal cortical dysplasia type Ia, IIa and IIb. Moreover, IL-17 and IL-17R levels directly correlated with the increased frequency of seizures in these patients. The authors found, by immunostaining, that IL-17 and IL-17R were highly expressed in dysmorphic neurons, neuronal microcolumns, astrocytes, balloon cells, and vascular endothelial cells, while NF κ B nuclear factor- κ B activator 1 and NF κ B-p65 were widely expressed in patients with focal cortical dysplasia. Thus, the authors concluded that IL-17 system overexpression and IL-17 signal transduction pathway activation may be involved in the epileptogenic process of cortical lesions in focal cortical dysplasia.

Janigro^[68] published a review on permeability across the epileptic barrier, challenging the theory that epileptic seizures can only be initiated by a foci of ‘epileptic’ neurons. After the recognition of astrocytic-neuronal communication, and the close crosstalk between astrocytes and brain

endothelial cells, it has been supposed that the BBB acts as a "neurovascular unit". These hypotheses led to new frontiers in epileptogenic research, focusing on investigations of cerebral blood flow, permeability of cerebral microvessels and leukocyte adhesion molecules at the BBB that have been proposed initiate pilocarpine-induced status epilepticus. Furthermore, BBB disruption itself was demonstrated to be a trigger-factor for seizures, with anti-convulsant effects obtained by administration of strong anti-inflammatory "BBB repair" drugs [69].

It is not known how systemic inflammation becomes active before an ictal event. It seems that pilocarpine, and other receptor-specific convulsive agents such as muscarin molecules, can activate the innate immune system by acting on T and B cells [69, 70, 71, 72, 73]. Furthermore, pilocarpine acts on leukocytes expressing muscarinic, cholinergic and nicotinic receptors and that muscarinic receptors are fundamental for CD8⁺ cytotoxic T-lymphocyte expression, as seen in experimental mice models with targeted deletions of each of the known muscarinic receptors (M1–M5). These studies have demonstrated that CD8⁺ T cells from M1 receptor-deficient mice were defective in differentiating into cytotoxic T lymphocytes [73]. These findings are therefore consistent with results linking pilocarpine-induced seizures to CD8⁺ T cell activation and mobilization from the spleen [73, 74]. Pilocarpine, when injected systemically, activates T cells to acquire a cytotoxic (CD8⁺) phenotype in the spleen; these events lead to an inflammatory response that via unknown pathogenic pathways causes BBB leakage in highly epileptogenic areas, such as the limbic system. It seems that in addition to T cells, other cell types are involved. It has also been demonstrated that pilocarpine-induced seizures, consistent with pilocarpine inflammatory action, are prevented by IL-1b antagonists or steroids [71, 72, 73, 74, 75, 76]. Furthermore, Costa-Ferreira *et al.* have recently found that transplantation of mononuclear bone marrow cells in murine models affected by pilocarpine-induced epilepsy effectively decreased seizure frequency and duration and levels of pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6. Moreover, transplants

performed at seizure-onset protected against pilocarpine-induced neuronal loss and gliosis and stimulated the proliferation of new neurons in epileptic rats [74].

In addition, the involvement of T-lymphocytes in epileptogenesis has also been demonstrated by Deprez-F *et al.* [77], who investigated the role of adaptive immunity in epileptic animal models by intravenous transfer of immunopurified T cells in mice lacking CD8⁺ T cells (β 2-microglobulin-knockout), CD4⁺ T cells (MHCII-knockout), or both (RAG1-knockout mice). It has been demonstrated that KAkainic acid, when injected in intra-hippocampal areas of adult mice, can cause a focal lesion in the CA1 area and hilus of the dentate gyrus and marked granule cell hypertrophy and dispersion leading to the onset of chronic focal seizures. These critical events are preceded by infiltration of T lymphocytes into the damaged tissue and macrophage-like cells into the dentate gyrus. The authors found that depletion of CD4⁺ and/or CD8⁺ T lymphocytes by targeted gene deletion results in decreased latency to seizure onset, consequently suggesting a key role for adaptive immunity in seizure onset. Furthermore, Deprez-F *et al.* [77] demonstrated that EEG analysis in mutant mice in which kainic acidKA was injected, showed that grafting of the missing T cell population, two days after the T cell transfer, had no influence on epileptogenesis, but strongly influenced F4/80⁺ macrophage-like cell infiltration in the dentate gyrus. Moreover, the authors found that macrophage recruitment was increased by CD8⁺ T cells in β 2-microglobulin-knockout mice, while CD4⁺ T cells transferred in MHCII-knockout and in RAG1-knockout mice blocked macrophage infiltration, thus causing decreased granule cell dispersion and survival and worsening the kainic acidKA-induced lesion. They concluded that intact adaptive immunity, T-cells and mononuclear phagocyte complex cross-talk have a key role in the control of neuronal integrity and survival in the damaged brain [77, 78].

However, Offner-H *et al.* demonstrated that central damage can also affect the systemic immune-response as well as peripheral inflammation which in turn could affect the neurons [79]. They showed that induction of stroke not only produces local ischemia and brain damage, but also has

profound effects on peripheral immune responses. They evaluated the effects on spleen and blood cells 4 days after stroke induction. They found a decrease in inflammatory cytokines in the middle cerebral artery occlusion-affected right brain hemisphere 96 hours after stroke onset compared with earlier time points [79]. They further demonstrated that stroke leads to splenic atrophy with a decrease of the organ size, a drastic loss of splenocytes and induction of annexin V+ and TUNEL+ cells within the spleen, with consequent reduction of T-cell proliferation responses and secretion of inflammatory cytokines. These changes produced a state of profound immune suppression, characterized by a drastic reduction in B-cell numbers in spleen and blood, and a novel increase in CD4⁺FoxP3⁺ regulatory T-cells [79]. The authors concluded that their results thus provided evidence to support the hypothesis that CNS damage caused by cerebral ischemia was able to cause a drastic state of immunosuppression, characterized by cell death as well as an increased presence of peripheral CD4⁺FoxP3⁺ regulatory T cells [79].

Taken together the above studies confirm that a state of neuronal inflammation could be the basic trigger for epileptic seizures; however, it is still unknown how a systemic immune imbalance could affect neuronal homeostasis and what kind of patients undergoes immune-mediated seizures. Moreover, the involvement of Th17 in the pathogenesis of "inflammatory seizures" may raise the speculation that Treg cells are indirectly involved in this process, although further studies are needed to clarify their role in epileptogenesis.

Cow's milk allergy and convulsive episodes: The description of our Paediatric case

S.J., a 40-day-old child, was admitted to our Paediatric Operative Unit, Vittorio Emanuele Hospital, University of Catania, Italy. Since he was 10 days old he had experienced frequent episodes of vomiting and diarrhoea, associated with episodes characterized by generalised clonic movements, with loss of consciousness, lasting about 5–10 minutes with spontaneous recovery. He was born at term (after 38 weeks gestation), small for gestational age

(weight: 1800 g and height 48 cm), by caesarean section, of a twin pregnancy. However, his twin died in utero during the 8th week of gestation.

At birth, the child was ~~hospitalised~~ hospitalized in the NICU Department of a nearby hospital, for intra-uterine growth failure (~~IUGF~~).

His physical examination revealed that his auxologic parameters were slightly under the normal range (weight: 3.100 g ($<3^{\circ}$ centile)), height: 49 cm (3° centile), head circumference: 36 cm (3° – 10° centile)). His neurologic exam showed the presence of generalized muscle hypertonia, alternating with phases of generalized hypotonia and marked rotula hyperreflexia.

At admission the child underwent an arterial blood gas analysis (~~ABG~~), which showed the presence of respiratory acidosis (pH: 7.31, PCO_2 : 38 mm_Hg, PO_2 : 51 mm_Hg, HCO_3^- : 19.2 mmol/~~L~~), with a transcutaneous O_2 saturation of 100%. During the hospitalization, he underwent serial measurements by blood gas analysis of his metabolic parameters, such as electrolytes, lactic acid and bicarbonate that were always within the normal range.

Routine blood tests (~~haematinics~~ with white cell count, urea ~~&-and~~ and electrolytes, renal and kidney function tests, immunoglobulins, ~~haematinics~~, stool and urine culture, Fehling test, blood ammonium and lactate and tandem mass test) were all in the normal range, with the exceptional presence of a slight anaemia (Hb: 10.3 g/dl). We therefore excluded the principal metabolic diseases associated with aminoacidemia, urinary organic acid, galactosemia and tandem mass.

During hospitalization, the child had recurrent episodes of vomiting after cow's milk consumption, often associated with leg tremors, episodes of hypertonia followed by hypotonic, retropulsion of his ocular globes and clonic movements of his inferior lip. Video-EEG showed a burst-suppression-like pattern with paroxysmal elements in bilateral temporal regions.

The child underwent further diagnostic exams, including ophthalmic specialist review, allergy specialist review, skin prick test for cow's milk protein, ECG, cardiac ultrasound scan, cerebral MRI that were all normal, with the exception of his chest X-Ray that showed the presence of: "a median large shadow of timic nature and marked broncho-vascular trauma".

His abdomen ultrasound scan showed the presence of "slight congenital left hydronephrosis".

Three days after his admission, we repeated a venous blood-gas-analysis that showed only little improvement (pH: 7.32, PCO₂: 54 mm_Hg, PO₂: 22 mm_Hg, HCO₃⁻: 23.2 mmol/L).

During the 10th day of hospitalization, the child's gastrointestinal symptoms persisted with the same frequency and intensity, and his neurological condition was unchanged, as far as his EEG was concerned. Therefore, as these episodes were often associated with the consumption of cow's milk, the child started an amino acid infant formula, lacking cow's milk protein (Neocate, Nestlé). After beginning this diet, his clinical condition abruptly improved, with resolution of his leg tremors and the episodes of alternating hypertonia and hypotonia. His blood gas analysis parameters also improved (pH: 7.36, PCO₂: 39 mm_Hg, PO₂: 51 mm_Hg, HCO₃⁻: 23.6 mmol/L), as well as his Video-EEG exam which was consistent with his chronologic age, with resolution of his pattern of neurological impairment.

One month after the onset of the amino acid infant formula, the child underwent a cow's milk protein challenge test, using an infant formula with cow's milk protein, but without galactose, in order to differentiate from any form of alteration in galactose metabolism. The challenge was performed according to the standard international protocols [1], and was positive, with episodes of vomiting and diarrhoea and the onset of suction movements when the child reached the dose of 150 mg of cow's milk. Moreover, during the challenge a Video-EEG was performed, and when the child consumed doses of between 35-mg and 70 mg of cow's milk, the exams showed a generalized slowing of his basal activity.

Due to the positive challenge test and the improvement of clinical, laboratory and EEG parameters after the cow's milk protein-free diet, a diagnosis of CMA cow's milk protein allergy was performed and the amino acid infant formula was continued.

At follow-up visit, the child was in good health, his auxologic parameters reached the 25[°]–50[°] percentile, and his parents did not report further episodes of vomiting and/or diarrhoea or neurological symptoms.

We described the above case for the peculiar clinical association between gastrointestinal symptoms in CMA cow's milk protein allergy, and neurological symptoms, presenting as seizures and alteration of muscle tone. As explained above, a possible common immune pattern could be the cause of this unusual association, perhaps due to some kind of immune disruption of the child's BBB. A figurative explanation of this hypothesis is shown in [Figure 1](#). An important consideration rising from this case is that the child was born from a twin pregnancy, in which the brother died at 8 weeks of gestation. This event may have caused an immune dysregulation towards the enhancement of pro-inflammatory processes and the production of oxidative stress that lead to [IUGR](#)^[A3].

Furthermore, a systemic pro-inflammatory immune response has been associated with a BBB disruption and this event would explain the association between the consumption of cow's milk and seizures after the onset of gastrointestinal symptoms. However, as there are no studies on the fetal immune response after a twin death, further studies are needed to clarify the pathogenic events leading to neuro-inflammation in this unusual pathological situation. It has been recently shown that intrauterine fetal death of one twin, of diamniotic twins, is associated with adverse perinatal outcome (lower Apgar score, lower birth weight, higher rates of preterm birth before 34 weeks of gestation) ⁸⁰. Furthermore, in case of fetal death in monochorionic twins, a higher rate of neurodevelopmental morbidity has been subsequently described ⁸¹.

This case could be considered a precursor leading to new frontiers of research on neuro-inflammation in atopic diseases.

Conclusions

Recent literature, experimental and clinical data are trying to explain the multi-system inflammatory state caused by cow's milk proteins, giving a reasonable explanation of extraintestinal phenotypic manifestations of CMA. In light of the established neurological manifestations of CMA, we suggest that the complex immunological mechanisms that regulate the allergic reaction may play a key-role in these epileptic manifestations in the context of CMA. Most current studies refer to IgE-mediated allergic reactions linked to epileptic seizures, whereas few data are available on the link between non-IgE-mediated reactions and neurological manifestations. Thus, in the causal diagnosis of epileptic seizures, when an allergic reaction is suspected, we suggest further investigation of both IgE-mediated and non-IgE-mediated reactions, considering this topic as a new frontier of research on neuro-inflammation in atopic diseases.

Expert commentary

Our review focuses on the neurological manifestations of CMAcow's milk protein allergy. The importance of our review is attributable to the diagnosis of this unusual presentation of CMA and a possible aetiological explanation, involving immune-mediated CNS damage that, through blood-brain barrierBBB disruption operated by pro-inflammatory cytokines, leads to neurological manifestations. It is not still clear if the immune component in the CNScentral nervous system is a response to peripheral inflammation or if these pro-inflammatory cytokines are locally produced. Nonetheless, we report evidence that CNScentral nervous system inflammation triggers seizures occurring in CMAcow's milk protein allergy. We believe that our review is innovative to highlight inflammation as a possible target for anti-convulsive treatment.

Five-year view

The importance of targeting inflammation as a trigger for seizures relies on the possibility of optimizing anti-convulsive therapy, driving research strategies towards anti-inflammatory immunotherapies. The actual research efforts are focusing on immunomodulatory therapies to decrease the inflammation in drug-resistant epilepsies, showing an increased efficacy in the treatment of these pathological entities, with less adverse reactions than anticonvulsive treatment itself. Therefore, the knowledge of the CNS inflammation in allergic diseases is fundamental to open new research topics for immunomodulating therapeutic strategies.

Key issues

The prevalence of food allergy, in the global population, accounts for 220–520 million affected people and represents a major global health burden. Usually, the gastrointestinal symptoms are ~~recognised~~ recognized as a “typical” presentation of the disease, with vomiting, diarrhoea and/or constipation and gastroesophageal reflux. Nevertheless, recently “atypical” symptoms, involving the ~~Central (CNS)~~ and the ~~Peripheral-peripheral Nervous-nervous Ssystem (PNS)~~ have been described. These symptoms can occur both in IgE- and non-IgE-mediated ~~cow's milk protein allergy (CMA)~~, as expression of a multi-system spread of immunogenic mechanisms that occur due to ~~cow's milk protein allergy CMA~~. After lymphoid follicles T and B cells activation, they migrate via the lymphatic and the circulation system to several target organs. This process is called “homing”. In a patient in whom tolerance is not achieved, T and B cells will be activated at a homing site by direct contact with a specific food antigen, releasing pro-inflammatory cytokines, antibodies and vasoactive peptides and causing an inflammatory reaction in the affected organ.

It could be speculated that an increased intestinal permeability, secondary to an inflammation, can cause the systemic passage of cow's milk allergens and by the “homing” process perpetuate a state of inflammation in other sites distant from the gastro-intestinal tract, such as the blood brain barrier-~~(BBB)~~. This mechanism would explain the unusual neurological manifestations, as recently described in the literature, in children affected by ~~cow's milk protein allergy CMA~~.

Hypotheses on a relationship between epilepsy and atopic diseases dates back to the first writings of Spangler RH, of the University of Philadelphia, who supposed that the mechanism “idiopathic

epilepsy” to be likely parallel in allergy and essential (nonorganic) epilepsy. These hypotheses are supported by papers that were published from this first assumption until recent research data.

It has been demonstrated that immune dysfunction and inflammation appear to alter the blood–brain barrier integrity, and it seems that the loss of this integrity, especially leukocyte endothelial adhesion may also be somehow involved in epileptogenesis. This arises from evidence that mast cells were considered the “immune gate to the brain”.

Taken together the studies contained in the review confirm that a state of neuronal inflammation could be the basic trigger for epileptic conditions, nevertheless it is still unknown how a systemic immune–imbalance could affect neuronal homeostasis and what kind of patients are susceptible to immune-mediated seizures.

In the causal diagnosis of epileptic seizures, when an allergic reaction is suspected, we suggest further investigation of the possibility of both IgE-mediated and non-IgE-mediated reactions. This topic is a potential new frontier of research on neuro-inflammation in atopic diseases.

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Table 1. Clinical studies on the evidence of a relationship between systemic gastrointestinal inflammation of various nature and seizures.

Type of study	Population	Population description	Analytic features	Results	Ref.
Prospective observational studies	Paediatric population	Reference population of 39,000 patients	Incidence study	Fourteen cases of GI associated with seizures. The authors estimated an incidence of gastroenteritis-related seizures of 1/ <u>out of</u> 10,000 children for afebrile seizures associated with gastroenteritis, with an earlier onset of symptoms with respect to the febrile seizures, and at a slightly higher age	28
Retrospective observational study	Paediatric population	Authors studied the electrophysiological features of these gastroenteritis-associated seizures using EEG on 25 paediatric patients	Observational study	Among the observed EEG, 32% were affected by focal, tonic or clonic seizures, 12% of focal with secondary ~~generalisation~~ <u>generalization</u> , 36% of ~~generalised~~ <u>generalized</u> tonic-clonic seizures and 20% consisted of staring only (absence-like) with no motor components, 52% of patients had clusters of seizures and 48% had isolated seizures. Interictal EEGs and brain imaging were normal for all patients. No patients required treatment with antiepileptic drugs	29
Retrospective cohort study	Paediatric population	323 children admitted to ~~Paediatric~~ <u>Emergency Room</u> for seizures	Observational study	Among the studied population, 247 (76%) had febrile seizure, 37 (12%) had unprovoked seizures, and 39 (12%) had non-febrile illness seizures. Children with non-febrile illness seizures were more likely than children with febrile seizures to have diarrheal illnesses accompanying their seizure (44 vs. 16%; p = 0.001). Frequency of cough, rhinorrhea, and rash did not differ significantly between children with	31

				febrile and non-febrile illness seizures	
Retrospective observational study	Paediatric population	400 asthmatic patients and 201 affected by idiopathic epilepsy	Prevalence study	Prevalence of 0.75% of idiopathic epilepsy among the 400 cases of asthma and prevalence of 0.79% and 0.73% of epilepsy in mild and moderate-to-severe asthma, respectively. Similar prevalence in general population	43
Case report	Paediatric population	3 children affected by cryptogenetic partial epilepsy	Observational study	Reduction of SCORAD in children with SPT [†] positive for CMA; Improvement of eczema;	44
Retrospective observational study	Paediatric population and their relatives	72 epileptic children and 202 controls	Prevalence study	Significant statistical higher rate of eczema in epileptic patient's mothers and rhinitis in their siblings with respect to controls	45
Perspective observational study	Paediatric population	56 children affected by Rolandic epilepsy	Observational study and therapeutic intervention	Non-allergic children displayed complete clinical and EEG remission in 29/39 cases (74%). The cow's milk-free diet made it possible to obtain clinical remission in 17/20 cases (80%) of the allergic group with normalization of the EEG. Less adverse events in the patients with no anticonvulsant therapy	46

CMA: Cow's milk protein allergy; GI: Gastrointestinal Inflammation.

Table 2. ~~experimental~~ Experimental studies on etiopathogenic hypothesis on peripheral and central inflammation triggering seizures.

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Type of study	Population	Population description	Analytic features	Results	Ref.
Prospective observational study	Animal ~~Model~~ model	Male rats treated with ~~trinitrobenzene-sulphonic-sulfonic-acid~~ TNBS and lipopolysaccharide during active colitis; ~~7-seven~~ rats as control group	Experimental study	At the peak of colitis, treated rats showed a disruption of circadian body temperature rhythm, as daytime fever followed by nighttime hypothermia. In response to ~~Lipopolysaccharide~~ lipopolysaccharide, treated animals had a significantly reduced fever and low plasma levels of IL-6 and TNF- α , while controls displayed a characteristic fever. During the phase of reactivation of colitis, treated animals did not show any fever or exhibit increased ~~of~~ IL-6 and TNF- α after lipopolysaccharide injection	~~18~~
Prospective ~~Observational-observational~~ study	Animal model	Adult rats in which authors administered an intracolonic administration of ~~2,4,6-trinitrobenzene-sulfonic acid~~ TNBS- (TNBS) to initiate a T helper-1 cell-mediated model of inflammatory bowel disease	Experimental study	Authors identified a microglia-dependent TNF α -mediated increase in CNS excitability associated with a phenotypical expression of increased frequency of seizures. The magnitude of the seizure susceptibility change was slightly weaker than what was observed after ~~2,4,6-trinitrobenzene sulfonic acid~~ TNBS-TNBS-induced inflammation. This phenomenon could be related to the incomplete diffusion to all brain areas of exogenous TNF- ~~alpha~~ such as amygdala and endorhinal cortex, both distant from the ventricles and	~~19~~

				involved in seizure generation	
Prospective observational study	Animal model	FcRgamma-deficient mice (group 1) and in FcRgamma-present mice (wild type B6) (group 2) in which hippocampal pyramidal cell death was induced by kainic acid (KA)	Experimental study	No activated microglial cells were detected 24 h after the KA injection in group 1, whereas many activated microglial cells were present in group 2. After 16 h the KA injection, in group 2 the production of nitrotyrosine as well as of the inducible nitric oxide synthase and cyclooxygenase-2 proteins. By contrast, the magnitude of oxidative stress was mild in group 1. Co-injection of a neutralizing antibody against FcgammaRII and FcgammaRIII with KA abolished pyramidal cell death and microglial activation	57
Prospective observational study	Adult population and animal model	Authors monitored the onset of seizures in patients undergoing osmotic disruption of BBB (blood-brain barrier) followed by intra-arterial chemotherapy to treat primary brain lymphomas and in naive pigs. Procedures were performed under barbiturate anaesthesia	Observational and Experimental-experimental study	Focal motor seizures occurred immediately after blood-brain-barrierBBB disruption in 25% of procedures and originated contralateral to the disrupted hemisphere. In a porcine model identical procedures generated identical results, and sudden behavioural and EEG seizures correlated with successful blood-brain-barrierBBB disruption	69
Prospective	Animal model	Fifteen laboratory	Experimental	Authors recorded EEG before,	70

observational study		rats	study	during and after hypertonic mannitol injection into the internal carotid artery. Rats' ~~blood-brain-barrier~~ BBB disruption was evaluated by gross anatomy or fluorescent microscopy. This disruption occurred in 20% of the rats and only under this circumstance acute EEG changes, recorded as brief EEG slowing, followed by a gradual increase in activity, were detected	
Prospective observational study	Animal model	Adult rats wild-type and perforin-deficient mice. Splenectomy was performed ~~prior-to~~ before pilocarpine injection	Experimental study	Splenectomy significantly reduced seizure-associated mortality. Histologic analysis of the spleens of rats exposed to pilocarpine revealed a significant increase in CD3 ⁺ and CD8 ⁺ T cells. Onset of status epilepticus (~~SE~~) and mortality were significantly decreased in perforin-deficient mice. The ~~blood-brain-barrier~~ BBB disruption damage was decreased in the perforin-deficient pilocarpine-treated mice	74
Prospective observational study	Animal model	Adult rats in which muscarinic receptor antagonist (atropine) and agonist (oxotremorine) were administered chronically through miniosmotic pumps	Experimental study	Oxotremorine stimulated, atropine inhibited the antibody and T-cell proliferative responses. Atropine suppressed the turpentine-induced leukocytic infiltration and tissue injury, and inhibited chemotaxis of leukocytes toward neutrophil and monocyte/lymphocyte chemoattractants	75
Prospective	Animal model	Twelve- to 14-	Experimental	Using immunohistochemistry for	77

observational study		week-old male wild-type and knock-out mice	study	CD45 ⁺ and CD3 ⁺ T-lymphocytes, authors showed microglial activation and infiltration of leukocytes in sclerotic tissue from patients with mesial temporal lobe epilepsy, as well as in a model of TLE (intrahippocampal ~~K~~ <u>Akainic acid</u> injection), characterized by spontaneous, nonconvulsive focal seizures	
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Figure 1. Diagram on the pathogenic hypothesis of the link between peripheral phlogosis induced by cow's milk protein allergy and CNS phlogosis triggering seizures.

APC: Antigen presenting cells; BBB: Blood-brain barrier. CMPA: Cow's milk protein allergy.

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