Immunology of febrile seizures

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ABSTRACT
Febrile seizures are the most common childhood neurological pathologies. They occur in 2–3% of children between 6 months and 5 years of age. A number of genetic mutations and also environmental factors contribute to their manifestation. The role of the immune system in the pathophysiology of febrile seizures has been raised since the end of 1980s. In children with febrile seizures the decreased production of immunoglobulins, particularly G2, was observed in brain with the normal serum level. In 1990 elevation of interleukin 1 beta in cerebrospinal fluid was found in the group with seizures as compared to the control group with the same infection but without febrile seizures. The association and significance of immunological disorders (increase of IL-1β, IL-6, IL-8, IL-1RA and decrease of CD4/CD8 level) in the manifestation of febrile seizures and especially prolonged febrile seizures was observed in the literature particularly during the last decade. Present studies suggest the correlation between immunological disorders and familial or sporadic incidence of febrile seizures. The studies on genetic polymorphism of interleukin 1β, 1α and IL-1 antagonist point to some associations between past febrile seizures and manifestation of temporal lobe epilepsy.

Key words: children, febrile seizures, cytokines, immunoglobulins, epilepsy

STRESZCZENIE
Drgawki gorączkowe stanowią najczęstszą patologię w neurologii dziecięcej. Występują u 2–3% dzieci w wieku pomiędzy 6 miesiącem a 5 rokiem życia. Ich ujawnienie się warunkuje szereg mutacji genetycznych i czynników środowiskowych. Od początku lat osiemdziesiątych ubiegłego wieku w patofizjologii drgawek gorączkowych podnosi się znaczenie układu odpornościowego. U dzieci z drgawkami gorączkowymi obserwuje się zmniejszoną produkcję immunoglobulin, zwłaszcza G2. W 1990 roku stwierdzono podwyższenie poziomu interleukiny1β w płynie mózgowo-rdzeniowym w grupie z drgawkami w porównaniu do grupy kontrolnej dzieci z tą samą infekcją, u których drgawki nie wystąpiły. Zainteresowanie problemami związku i znaczenia układu immunologicznego w ujawnieniu drgawek gorączkowych, a zwłaszcza drgawek gorączkowych długotrwałych obserwuje się w piśmiennictwie przedmiotu, zwłaszcza w ostatniej dekadzie (zwiększenie poziomu IL-1β, IL-6, IL-8 i obniżenie CD4/CD8). Współcześnie badania wskazują na istniejące korelacje między zaburzeniami immunologicznymi a rodzinnym lub sporadycznym występowaniem drgawek gorączkowych. Badania nad genetycznym polimorfizmem interleukiny 1β, interleukiny 1α antagonisty receptora IL-1 zwracają uwagę na związki między przebyciem drgawek gorączkowych a ujawnieniem się padaczki płata skroniowego.

Słowa kluczowe: dzieci, drgawki gorączkowe, cytokiny, immunoglobuliny, padaczka

Febrile seizures (FS) are the most frequent neurological pathologies in children. Polish epidemiological studies indicate that 2.9% of children experience FS [1]. Both genetic and environmental factors are important for their manifestation. The cooperating nervous, immune and endocrine systems take part in the preservation of organism integration in changing conditions of external and internal environment. The role of the immune system in FS has been raised since the 1990s [2–4]. Particular attention was paid to the role of pre- and anti-inflammatory cytokines in experimental models in animals and also in children with FS. Despite a significant progress in genetic studies on FS, the etiology of this pathology has not been recognized satisfactorily, particularly in sporadic cases. Current studies indicate that the role of the immune system can explain some aspects of FS pathogenesis.

The immune system influences the nervous system via cytokines produced by macrophages and lymphocytes. Cartmell et al. [5] demonstrated that interleukin receptors, e.g. IL-1, TNF, IL-6 are found in numerous brain sites, particularly in the hippocampus, already in the early stage of development. Hippocampal sites containing cytokine receptors are involved in temperature regulation. Experimental studies of Cartmell et al., [5] who administered interleukin-1 antagonist into dentate gyrus and CA3 region of the hippocampus, proved delay of febrile response after application of lipopolysaccharide to animals in the model of FS.
Cytokines and Temperature Regulations

Cytokines act as endogenous pyrogens, mainly in monocyto-type cells, and their release is a significant triggering factor controlling febrile relations and further phases initiating an acute inflammatory response. Saper [6] summing up the effect of fever on the manifestation of seizures, emphasized that a number of neuronal processes essential for provoking and evoking seizure episodes depend on the body temperature. Mainly they include: transport through cell membranes (passive and through ion channels), metabolic pump, action potential, release and reabsorption of neurotransmitters, neuronal synchrony and neuronal survival. The effect of interleukin-1 is manifested first of all by the elevated body temperature. Lipopolysaccharide, a component of the cell wall of gram-negative bacteria, is an external pyrogen in bacterial infections. In the study of Heida et al. [7], lipopolysaccharide-induced hyperthermia was in experimental animals a set point for a model of febrile and epileptic seizures. Peripheral administration of LPS stimulates macrophages to the production of pro- and anti-inflammatory cytokines: tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), prostaglandin E-2. Saper [6] summed up their mechanism of activity suggesting that these cytokines act through endothelial cells of circumventricular organs (CVO) and also through vagal afferent pathways stimulating the activity of the enzyme cyclooxygenase-2 (COX-2), which then catalyzes the conversion of arachidonic acid into prostaglandin E2. PGE2 then acts in the hypothalamus on thermoregulatory pathways causing an increase in body temperature. As experimental studies of van Dam et al. [8] have demonstrated, cytokines are produced not only in the periphery but also by microglia, astrocytes and some neurons in the central nervous system. In 1998 Löschler and Siemens [4] found in children who experienced febrile seizures, an elevated level of PGE2 in cerebrospinal fluid as compared to children with fever without febrile seizures and also to a group of aphyretic children. The results of this study carried out on patients with febrile seizures were confirmed by experimental studies indicating the elevated level of pro- and anti-inflammatory interleukins in animals with hyperthermia-induced seizures.

Interleukin 1 Beta and Febrile Seizures

In 1990 Helminen and Vesikari [2] were the first to show the enhanced interleukin 1β production by peripheral mononuclear cells extracted from children with febrile seizures after stimulation with lipopolysaccharide from gram-negative bacteria as compared to other children with the same infection but without seizures. The studies on the mechanism in which interleukins influence febrile seizures in immature subjects with a genetically reduced seizure threshold demonstrated the involvement of both excitatory (glutamatergic) and inhibitory (gabaergic) systems. Vivani et al. [9] in their experimental study showed that hippocampal cells exposed to interleukin-1 beta demonstrated the increased calcium influx when exposed to NMDA. The authors explained that this increased calcium influx was due to phosphorylation of the NR2A/B subunit of the NMDA receptor. Most probably this mechanism is similar in children and it leads to fever-induced increased excitability. An experimental study by Wang et al. [10] showed that IL-1β receptors of rich density can be found on granule cells and their dendrites in the direct area of NMDA receptors. IL-1β manifests both inhibitory and excitatory effect on glutamatergic transmission. High concentration of interleukin-1β (micromolar) causes the effect of neural excitation and the low concentration has an inhibitory effect. Interleukin-1β also manifests inhibitory effect on gabaergic transmission. Wang et al. [10] showed that this interleukin decreases the gabaergic transmission in hippocampal neurons. Gabaergic inhibition also depends on the interleukin concentration and can be blocked by interleukin-1β receptor antagonist (IL-1ra). Thus, experimental studies suggest the role of interleukin-1 beta in the genesis of febrile seizures by the increase of excitation and the decrease of inhibition [10]. Additionally, Wilkinson [11] and Landgraf [12] also demonstrated the role of interleukin-1 beta in seizure excitability resulting from its influence on vasopressin and oxytocin release. Interleukin-1 beta is synthesized in brain not only under the effect of external pyrogens but also as the result of experimentally-induced (kainic acid) seizure events, particularly in prolonged seizures [13]. There comes to the induction of IL-1 beta secretion in the time shorter than two hours from the onset of the seizure activity and this secretion drops to the initial level after seven days. Thus, investigations on the level of pro- and anti-inflammatory interleukins should take into account duration of seizures and the time elapsed from the last seizure. The latest reports [14] concerning the level of individual pro- and anti-inflammatory interleukins in children with seizures confirmed the earlier experimental and clinical findings of Ichiyama et al. [3] on the correlation between the duration of a seizure episode and the level of interleukins. Asano et al. [14] found a significantly higher level of interleukin-8 after episodes lasting longer than 24 hours than after episodes of typical febrile seizures.

Current Studies on the Effect of Cytokines in Febrile Seizures

Current studies on the effect of cytokines in febrile seizures are focused on three fundamental problems: the clinical significance of individual interleukins, whether there exists genetic polymorphism related to individual pro- and anti-inflammatory interleukins and whether it can be a marker of special susceptibility to febrile seizures and the possibility of the development of epilepsy, whether the elevated level of some interleukins plays a protective role against ischaemic excitotoxic brain damage in pathologies with prolonged seizures, including also children with prolonged FS.

Other Pro- and Anti-Inflammatory Cytokines and Febrile Seizures

In the years 2002–2010 were published a few further studies indicating the role of individual interleukins in experimental models and clinical trials in children with FS. Apart from interleukin-1 beta, mainly the interleukin-6 and tumour necrosis factor alpha (TNF-α) demonstrate pro-inflammatory properties. Interleukin-1 receptor antagonist (IL-1RA) and interleukin-10 are anti-inflammatory cytokines demon-
stratifying negative feedback effect during the febrile response. In the regulation of inflammation, the balance between pro- and anti-inflammatory cytokines is more critical than a single cytokine concentration. The study of Dinarello [15] demonstrated that 100-fold molar excess of IL-1RA was needed to prevent the response to IL-1 [15]. IL-1RA rapidly reduces seizure activity in experimental models. The analysis of different brain regions affected by seizures using c-fos mRNA as a marker suggests that IL-1RA reduces seizures by inhibiting their generalization from the hippocampus to the motor cortical areas. The studies of Virta et al. [16] related to the serum level of pro- and anti-inflammatory cytokines in children with FS demonstrated high positive correlation between the diagnosis of febrile seizures and the value of IL-1RA/LI 1-beta ratio [16]. Its importance was confirmed in the regression analysis together with the increased level of IL-6 as a factor significantly characterizing the group of children with FS [17]. In recent years, the studies of Fakuda et al. [17] confirmed that interleukin-6 plays an anti-convulsive role in experimental hyperthermia-induced seizures on models of FS in children. These authors administered nasally recombinant human IL-6 to experimental animals. In the group exposed to hyperthermia the latency in relation to the appearance of seizure discharges was statistically significantly longer and the duration of seizures was significantly shorter. The authors concluded that IL-6 plays an anti-convulsive role in experimental animals, which might suggest similar properties of this cytokine in children with febrile seizures. Earlier studies of Biber et al. [18] demonstrated that stimulation of astrocytes and brain slices of cortex with IL-6 induced adenosine A1 receptor mRNA which is a powerful endogenous anti-convulsive substance [18]. Similar mechanism in immature brain can be induced in children with febrile seizures causing an IL-6 level increase in cerebrospinal fluid and serum [16] and in children with epilepsy [19].

**CYTOKINE GENETIC POLYMORPHISM AND FEBRILE SEIZURES**

Genes responsible for cytokine production and factors affecting their expression can contribute to the aetiopathogenesis of febrile seizures. In recent years the studies have demonstrated that cytokine gene polymorphism is associated with different immunoinflammatory diseases such as: asthma, rheumatoid arthritis, atopic dermatitis, thyroid diseases or multiple sclerosis [20]. In the year 2000 Kannemoto et al.[21] demonstrated genetic polymorphism of interleukin-1 beta, 1 alpha and also IL-1 receptor antagonist in adult patients with temporal lobe epilepsy. This polymorphism was predominant in drug-resistant epilepsy. The associations between drug-resistant temporal lobe epilepsy and experienced FS have been the subject of interest for years [23]. The authors suggest some clinical importance the polymorphism of interleukin-1 beta can demonstrate in children with FS. It might be a kind of clinical marker of drug-resistance in temporal lobe epilepsy appearing in a small group of children who earlier experienced focal and prolonged febrile seizures [21,24]. In 2002 Virta et al. [24] reported an increased frequency of interleukin-1 beta genetic polymorphism at the position 511 allele 2 in children with febrile seizures. These alleles are associated with the increased production of interleukin-1. Children with this polymorphism manifest increased pro-inflammatory reaction in the period of febrile disease.

Several genetic and clinical studies have been carried out since 2002 in the search for genetic polymorphism in relation to interleukins. IL-4 is a cytokine with anti-inflammatory properties, and it inhibits the production of intraglobins IL-1, IL-6 and tumour necrosis factor. The studies of Tsai et al. [20] did not confirm that polymorphisms concerning intron-3 could be a useful marker for the susceptibility to febrile seizures. To date, the interleukin polymorphism has not been analysed in correlation with the most frequent types of clinical febrile seizures such as: sporadic cases (most frequent) with multiple recurrence from an early period of 1–2 years and regressing to the end of the 5 year of life, familial (simple seizures) and also focal, prolonged seizures (complex seizures). These correlations may be of clinical importance as children with complex seizures more often develop epilepsy already by the end of the first decade of life [1]. In 2005 investigations were initiated on the determination of some correlation between genetic susceptibility to the occurrence of individual types of seizures and the polymorphism of interleukin-1 beta but they are only of fragmentary nature [25]. The study population consisted of 168 children with simple seizures (60 familial and 108 sporadic cases), 68 with complex seizures and 158 healthy children. The frequencies of polymorphisms – 31 C/T and -511 C/T were investigated [25]. In these studies a significant polymorphism was associated only with sporadic (not familial, not complex) cases. These polymorphisms had already been investigated several times in children with febrile seizures but without division into the types of febrile seizures. Their significance was demonstrated in one study [16] but in two studies this significance was not shown [26,27]. A hypothesis that genetic susceptibility to sporadic seizures depends on different genetic mechanisms compared to the susceptibility observed in children with familial seizures and febrile seizures can be the only explanation of the results obtained by Kira et al. [25]. Clinically, also sporadic cases differ from familial cases which can be well proved in prospective long-term studies [1]. Sporadic cases have a clinically milder course, and the recurrence is rare as well as pathological changes on EEG, seizures lasting >30 min. are also less frequent. Family history of FS has an influence, besides familial prevalence of epilepsy, on the appearance of generalized epilepsy among children with febrile seizures [1]. FS are more frequent in children more often suffering from diseases accompanied by fever (more than 4 times a year) and in children exposed to infections in a nursery [28]. It may be assumed that environmental factors such as exposure to viral infections and host defence related to cytokine gene polymorphism can be the cause of sporadic cases of febrile seizures and can play a significant role in their manifestation. Undoubtedly, the small number of studies concerning cytokine gene polymorphism, particularly in Caucasian population, makes it difficult to express explicitly the hypotheses or concepts.
CYTOKINES AND PROLONGED FEBRILE SEIZURES AND ENCEPHALOPATHY WITH FEBRILE CONVULSIVE STATUS EPILEPTICUS

Recently, the studies have been undertaken on cytokines in children with prolonged FS to demonstrate the role of pro- and anti-inflammatory cytokines in other episodes than typical FS [28,29]. Prolonged FS (longer than 30 min.) are observed in about 15% of all children with complex FS [1].

Most of these cases are admitted to paediatric hospitals and they require specialist therapy in the paediatric neurology or intensive care units. Sometimes the children present the symptoms of acute encephalopathy with febrile convulsive status epilepticus (AEFCSE). The clinical symptoms of this encephalopathy are associated with excitotoxicity-related ischaemia. Takashashi et al. [30] worked out the diagnostic criteria for this encephalopathy. They include: occurrence of seizure or cluster of seizures longer than 30 min. within 24 h after the onset of fever, manifestation of subcortical white matter lesions on diffusion-weighted MRI at day 1–5, disturbance of consciousness, hemiparesis, second seizures after 1–5 days (only in part of the patients), no positive cultures (bacteria, fungi, viruses). This type of encephalopathy can be diagnosed when no other causes of brain damage were found (vascular, toxic, metabolic, endocrine or drug-induced disorders). Ichiyama et al. [28] determined the level of several cytokines in prolonged FS and AEFCSE. After prolonged seizures and in prolonged febrile seizures without encephalopathy, the level of IL-6 (but not IL-10) was elevated significantly higher in encephalopathy than in prolonged FS. Analysing the results of the study the authors suggested that in these cases, the main role of IL-6 is to protect neurons in ischaemia which is a pathogenetic picture of seizure-induced encephalopathy and excitotoxic damage following prolonged FS. Experimental studies of Yamashita et al. [31], which demonstrated that the administration of an anti-IL-6 receptor antibody increased the infarct size after middle cerebral artery occlusion, correspond well with the above described studies [31]. The role of cytokines in febrile seizures has not been recognized satisfactorily yet. Most probably, cytokine polymorphism is, at least in part, an aetiopathogenetic factor in the manifestation of FS, particularly in sporadic cases. Interleukins also play a role in the protection of neurons against ischaemia and cytotoxic damage in prolonged FS. To date, there have not been explicit evidence confirmed by greater number of experimental and clinical studies, whether cytokine genetic polymorphism can be a risk factor of the development of epilepsy in children with febrile seizures.

ASSESSMENT OF SPECIFIC IMMUNITY IN CHILDREN WITH FEBRILE SEIZURES

Specific immunity disorders may be caused by abnormalities in maturation, differentiation and activation of haematopoietic stem cells, thymic cells, T- and B-lymphocytes and also by abnormalities in the mediators indispensable in these processes, such as earlier described cytokines. It has been assumed that the role of cytokines in FS is associated with their improper secretion and/or improper differentiation or functioning of membrane receptors in neurons. Interleukins induce B-lymphocytes to produce and secrete immunoglobulins. The immune system disorders are genetically conditioned or caused by external factors, e.g. repeated infections. Immunological problems concerning the function of cellular and humoral elements have been relatively rarely raised in patients with febrile seizures [32–35], but slightly more frequently in epileptic children [35–38]. In these studies, the decrease of the level of CD4 lymphocytes, the increase of CD8 level and the decrease of CD4/CD8 ratio [34], reduced IgG2 [39] and IgA levels [40] were observed in children with febrile seizures. Classic studies concerning this subject were published by Olofsson et al. [38]. The first study of this author published in 1982 was related to immunoglobulin abnormalities in children with febrile seizures [32]. In 42% of children with febrile seizures intrathecal synthesis of at least one immunoglobulin (IgA, IgM) was observed in the acute phase at admission and in 25% at the follow-up examination after 4 weeks. However, Lentini et al. [39] found the lower level of IgG2 in children with febrile seizures than in the controls and Isaacs et al. [40] detected serum IgA level nearly the same as in the control group.

The decrease of IgA level in patients with FS and in untreated patients with epilepsy is thought to correlate with HLA-A1 system. Direct activity of viruses e.g. caused by HHV-6 infection in relation to memory T-lymphocytes (CD4/CD45RA) can be another mechanism of dysregulation of β-lymphocytes activity in febrile seizures [41,42]. The influence of a virus on T-lymphocyte disturbs the balance of immune response induction and suppression factors which in consequence leads to the disorder of B-lymphocytes activity. So far, in the studies on specific humoral response in children with FS the results have not been analysed comparatively in groups with single, sporadic and multiple seizures which are often of familial origin. Zubiel [35] proved the most profound disorders in quantitative evaluation and function of B- and T-lymphocytes subpopulation in children with single fit of FS, which were often sporadic.

The children in both groups analysed by the author did not differ as regards the frequency of infections, sex and age. The material for tests was collected after infection regression. In the group of children with FS the author found a lower level of virgin (CD4RA) and memory (CD4/RO) T-lymphocytes in relation to healthy children. Testing the function of T-lymphocytes (by determining receptor CD4OL expression before and after PHA stimulation and lymphocyte proliferation in response to pokeweed mitogen action as well as a stimulation index) demonstrated that in single (sporadic) FS, T- and B-lymphocytes proliferative capability was found to be decreased not only in relation to the group of healthy children but also in relation to the children with multiple febrile seizures. The author also demonstrated that the lymphocyte dysfunction leads to the decrease of the concentration of total IgG and IgG2 and IgG4 subgroups and kappa and lambda light chains in the group with single FS as compared to the group with multiple FS. However, in the latter group, the level of IgG1, IgG2 and IgG4 was also lower in comparison to the control group of healthy children. In the mentioned studies from
the year 2000, also higher concentration of B-lymphocytes with CD5 marker was demonstrated in children with single seizures. Earlier studies indicated that the increased number of B-cells with CD5 marker can be observed in autoimmune diseases, and natural antibodies are produced by this subpopulation of lymphocytes B. Thus, it may be assumed that autoimmune disorders are in the background of the most frequent syndrome of febrile seizures without recurrence.

Current studies on the immune system in children with FS have suggested their congenital nature and genetic differences between the fit of classical FS with sporadic discharge and multiple (familial) seizures. In the case of children with frequent events of FS, the observed abnormalities can point to the existence of immune system activation induced by repeated episodes of FS.

Further investigations concerning the relation between immunological system (humoral, cellular response, cytokine genes polymorphism) and the type, course and prognosis in febrile seizures are needed. Data provided by these studies could be used in paediatric neurologist’s clinical practice.

REFERENCES


