The Role of Folate Receptor Autoimmunity in Cerebral Folate Deficiency

T. Opladen¹,², N. Blau¹, V. Th. Ramaekers²

¹Division of Clinical Chemistry and Biochemistry, University Children’s Hospital Zürich, Switzerland
²Division of Pediatric Neurology, Dept. Pediatrics, University Hospital Aachen, Rheinisch-Westfälische Technische Hochschule, Aachen University, Aachen, Germany

Abstract

For normal functioning, the central nervous system requires folate stores which depend on folate homeostasis and intact transport mechanisms across the choroid plexus. Cerebral folate deficiency (CFD) describes a neurological syndrome associated with low cerebrospinal fluid (CSF) 5-methyltetrahydrofolate (5MTHF) in the presence of normal folate metabolism outside the nervous system. Idiopathic cerebral folate deficiency was found in a number of patients presenting with microcephaly, irritability, psychomotor retardation, cerebellar ataxia, spastic paraplegia, dyskinesias, and occasional seizures. Treatment with folinic acid proved beneficial in most patients. Serum analysis from 25 out of 28 analysed children with CFD revealed blocking autoantibodies to the membrane-bound folate receptors. All 28 serum samples of age-matched controls were negative. Cerebral folate deficiency is more common than initially assumed and should be corrected as early as possible by oral administration of folinic acid. The finding of blocking autoantibody against the folate receptor at the choroid plexus provides an autoimmune explanation for this hitherto underestimated and treatable condition.

Introduction

Folic acid is a water-soluble vitamin that functions as one-carbon donor in various metabolic cycles. It is involved in the biosynthesis of thymidylates and purines, the methionine synthesis via homocysteine remethylation, the methylation of phospholipids, the serine and glycine interconversion, and the metabolism of histidine and formate. It is therefore essential for growth, reproduction, maintenance of normal body function and brain development in childhood [1, 2]. Twenty-five different compounds are referred to as the folates. They can be interconverted enzymatically, but the natural active form in serum consists mainly of 5-methyltetrahydrofolate (5MTHF). Systemic folate deficiency is associated with macrocytic anaemia, high blood levels of homocysteine, and neural tube defect in newborns [3, 4]. In contrast cerebral folate deficiency (CFD) has been defined as any neurological syndrome associated with low cerebrospinal fluid (CSF) 5-methyl-tetrahydrofolate...
(5MTHF), the active folate metabolite, in the presence of normal folate metabolism outside the nervous system [5].

Recently differentiation of CFD in a primary (“idiopathic”) form and a secondary form was suggested [5]: The primary form becomes manifest from the age of 4 months, starting with marked unrest, irritability, and sleep disturbances followed by psychomotor retardation, cerebellar ataxia, spastic paraplegia, and dyskinesia; epilepsy developed in about one third of the cases. Most children showed deceleration of head growth from the age of 4 to 6 months. Visual disturbances and progressive sensorineural hearing loss are not invariably present but may develop from the age of 3 years and 6 years, respectively. The clinical findings are summarised in figure 1. Neuroimaging does not show any specific findings and was normal in half of the children whereas the other children showed atrophy of frontotemporal regions and periventricular demyelination or slowly progressive supra- and infratentorial atrophy [5].

![Figure 1](image-url)

**Figure 1:** Overview of the main clinical features in patients with primary CFD correlated to the age of onset: CFD patients develop normally during the first four months. Characteristic symptoms start with marked unrest, irritability, sleep disturbances and deceleration of head growth from the age of 4 to 6 months followed by psychomotor retardation, cerebellar ataxia, spastic paraplegia, and dyskinesia. Epilepsy developed in about one third of the cases from the age of one year. Visual disturbances and progressive sensorineural hearing loss are not invariably present but may develop from the age of 3 years and 6 years, respectively. (Reprinted with permission from [5] and [12]).

Secondary forms of CFD have been recognised during chronic use of antifolate and anticonvulsant drugs and in various known conditions such as Rett syndrome, Aicardi-
Goutières syndrome, 3-phosphoglycerate dehydrogenase deficiency, dihydropteridine reductase deficiency, aromatic amino acid decarboxylase deficiency, and Kearns-Sayre syndrome [6, 7, 5].

Oral treatment with 5-formyltetrahydrofolate (folinic acid) resulted in a favourable clinical response in idiopathic and secondary CFD patients. Treatment should be started as soon as possible in low doses at 0.5–1 mg/kg/day since patients identified before the age of six years showed a better outcome than patients treated after the age of six years. Dose has to be titrated individually, since some patients require higher daily doses of folinic acid at 2–3 mg/kg/day to normalise CSF 5MTHF values [5]. Careful clinical, laboratory and EEG follow-up should be performed continuously during treatment to prevent over- or underdosage.

The exact pathophysiology in CFD remains unclear but based on the knowledge of normal peripheral folate homeostasis, CFD could be a consequence of either disturbed folate transport to the central nervous system (CNS) or an increased folate utilisation and catabolism within the CNS. The most important folate transport mechanisms in humans are the reduced folate carrier 1 (RFC1) and the family of folate receptor proteins (FR) which possess different binding properties and are distributed at various sites in normal human tissues and are highly expressed in a variety of cancer cells [8]. RFC1 is ubiquitously distributed and represents a low-affinity folate transporting system with bidirectional transport across cellular membranes. In contrast the folate receptors are high-affinity proteins which function at the physiological nanomolar range of extracellular folate concentrations. FR1 is mainly distributed at epithelial cells, such as choroid plexus, lung, thyroid, and renal tubular cells, while FR2 is mainly located within mesenchymal derived cells, such as red blood cells. For passage across the blood-CNS barrier 5MTHF is bound by the FR1, anchored to choroid epithelial cells and followed by endocytosis, storage, and subsequent delivery to the spinal fluid compartment where it will be transported into neuronal tissues (figure 2). A specific disorder of folate transport across the choroid plexus was described for the first time in an adult male with a slowly progressive neurological disease characterized by a cerebellar syndrome, distal spinal muscular atrophy, pyramidal tract dysfunction, and perceptive hearing loss [9]. A diminished expression and/or secretion of FR1 and impaired folate release from its binding site were suggested but the exact cause remained unclear.

To exclude genetic alterations of FR1 or FR2 in our patients the folate receptor genes were analyzed by DNA sequencing and found to be normal. An alternative possibility is that impaired folate transport across the blood-CSF barrier is caused by circulating serum autoantibodies that block the binding of folate to the receptor. Such blocking autoantibodies were found in serum from women with a pregnancy complicated by a neural tube defect [10]. We therefore tested serum from patients with idiopathic CFD for the presence of autoantibodies to the folate receptor.
Figure 2: Schematic presentation of the active transport for folates across the blood-CSF-barrier localised at the epithelium of the choroid plexus.

The folate receptor protein 1 (FR1) binds and incorporates 5-methyltetrahydrofolate. 5MTHF is converted to folyl-polyglutamate by the action of folylpoly-gamma-glutamate synthetase (1). Stored folylpolyglutamate can be converted again to its monoglutamate form by gamma-glutamyl-hydrolase (2) after which the monoglutamate form of folate can leave the choroid plexus to the spinal fluid compartment. Expression of FR1 is correlated inversely with extracellular folate concentrations. (Reprinted with permission from [5], [7] and [13]).

Patients and methods

After exclusion of other neurodegenerative diseases and inborn errors of metabolism 28 patients (20 boys and 8 girls; median age, 7.1 years, range 2.5 to 19.3) fulfilled the clinical criteria of cerebral folate deficiency with normal levels of serum and erythrocyte folate. The births and neonatal histories of these patients and the pregnancies of their mothers had been normal except in the case of one child, who had been born prematurely, at 28...
weeks of gestation. All the parents were healthy and unrelated except for the parents of one patient, who were first cousins.

A lumbar puncture was performed according to a standardized protocol. 5MTHF was measured with HPLC using electrochemical detection and compared with values derived from 99 normal controls, as previously described. [11, 6]. After the diagnosis had been established, treatment with folic acid (0.5 to 1 mg per kilogram of body weight daily in two divided doses) was started according to treatment protocol [5]. The history and neurological examination of the 28 age-matched control subjects (17 boys and 11 girls; median age, 7.6 years; range, 1.9 to 19.0) did not reveal any of the symptoms of the cerebral folate deficiency syndrome [12].

Serum samples of patients, age-matched controls and five mothers of patients with CFD were kindly analyzed for autoantibodies against the folate receptors in the laboratory of the Department of Medicine and Biochemistry, State University New York according to the procedures published previously [10]. Additionally serum from 41 subjects with central nervous system disease unrelated to CFD was examined [12].

Results

CSF analysis confirmed the clinical diagnosis of CFD showing reduced 5MTHF concentration in all patients compared with normal reference values (Figure 3/Table 1). In nine patients CSF analysis showed a reduced concentration of 5-hydroxy-indoleacetic acid (5HIAA) in the presence of normal homovanillic acid (HVA) concentrations. Six patients had isolated reduction of neopterin (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HVA (nmol/l)</th>
<th>5HIAA (nmol/l)</th>
<th>HVA/5HIAA</th>
<th>3OMD (nmol/l)</th>
<th>5HTP (nmol/l)</th>
<th>L-DOPA (nmol/l)</th>
<th>Neo (nmol/l)</th>
<th>Bio (nmol/l)</th>
<th>5MTHF (nmol/l)</th>
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<td>CFD</td>
<td>28</td>
<td>n</td>
<td>n (↓)</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n (↓)</td>
<td>n</td>
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<tr>
<td></td>
<td>28</td>
<td>245–537</td>
<td>58–207</td>
<td>1.9–5.2</td>
<td>11–79.5</td>
<td>1.6–12</td>
<td>5.2–14</td>
<td>5.9–20.5</td>
<td>14.5–20.5</td>
<td>11.4–38.7</td>
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Table 1: Overview of the CSF analysis in 28 patients with CFD: All patients showed reduced CSF 5MTHF concentration. One third of CFD patients had further a reduced concentration of 5-hydroxy-indoleacetic acid (5HIAA) in the presence of normal homovanillic acid (HVA) concentrations. Six patients had isolated reduction of neopterin (Reference values from [11]). (reprinted with permission from [12]).
Figure 3: 5MTHF concentrations in cerebrospinal fluid from 28 patients with CFD compared to the age-related reference values (grey shaded area)

Blocking autoantibodies against the folate receptors were identified in serum specimens from 25 of 28 children with cerebral folate deficiency and in 0 of 28 matched control subjects (P < 0.001 by the chi-square test) (table 2). No autoantibodies against the folate receptors were detected in serum from 5 mothers of children with CFD and 41 subjects with central nervous system disease unrelated to this syndrome. The mean titer of blocking autoantibodies in the serum of cerebral folate deficiency subjects was 0.87 pmol of folate receptor blocked per milliliter of serum (table 2). The mean apparent Ka for the binding of these autoantibodies to the folate receptor was 5.54 x 10^{10} liters per mole [12]

Serum specimens from three children with cerebral folate deficiency did not contain these autoantibodies. Patient 9, who had four of the clinical criteria for the syndrome, had frank autistic behaviour and recovered completely after receiving a daily dose of 400 µg of folic acid contained in a multivitamin formula; he currently attends a regular school. He was the first child identified to have the syndrome and received a multivitamin containing folic acid, whereas all the other children were treated with folinic acid. Patients 7 and 21 also had remarkable improvements with folinic acid, although the changes were not as dramatic as those in Patient 9.

**Discussion**

Idiopathic cerebral folate deficiency (CFD), defined as isolated lowering of CSF 5MTHF in the presence of a normal systemic folate pool and metabolism, is more common than initially assumed. After the first report on five children [13], a total of thirty-one children are known with the typical uniform history and clinical phenotype. CFD is further associated with autism and shows in some patients very dramatic response to folinic acid supplementation [5, 12, 14]. So far the exact cause for the clinical and biochemical finding were
unknown, although already at the first report a disturbed folate transport was suggested [13].

The finding of blocking autoantibodies against folate receptors in serum from children with infantile-onset cerebral folate deficiency supports the hypothesis that this neurological syndrome can be a consequence of autoantibody-impaired folate transport into the cerebrospinal fluid. The high affinity of the autoantibodies (mean $K_a$, $5.54 \times 10^{10}$ litres per mole) allows them to prevent folate from binding to the receptors on the epithelial cells of the choroid plexus. Since autoantibodies with a mean $K_a$ of $2.2 \times 10^{10}$ liters per mole were shown to block the binding and cellular uptake of $[^3H]$folic acid by KB cells [10], autoantibodies with a higher $K_a$, such as those in the serum from subjects with cerebral folate deficiency, would have a similar effect. Autoantibodies against GPI-anchored folate receptors preferentially bind to epithelial cells on the plasma side of the choroid plexus. Folate receptors in the lungs, kidney and thyroid gland might also be affected by these blocking autoantibodies. But recent studies could show that tissue FR expression within lung tissue is limited to the apical membrane of the epithelial cells. This means that in the blood circulating antibodies cannot gain access to them [8]. In kidneys, the folate receptors on the luminal side of the proximal renal tubules will not be affected because immunoglobulins do not pass into the renal tubules of normal kidneys.

The positive effect of treatment with folinic acid in patients with CFD remains unexplained. Different mechanisms are possible: First, treatment with pharmacologic doses of 5-formyltetrahydrofolate (folinic acid), most of which is enzymatically converted in vivo

<table>
<thead>
<tr>
<th>Patients with Cerebral Folate Deficiency</th>
<th>Age-matched healthy controls</th>
<th>Mothers of patients with Cerebral Folate Deficiency</th>
<th>Patients with central nervous system disease unrelated to CFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>28</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>Blocking autoantibodies detected in number of subjects</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Titer* (pmol receptor blocked/ml)</td>
<td>0.87 (0–1.55)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Affinity constant* (litres/mole)</td>
<td>$5.54 \times 10^{10}$</td>
<td>–</td>
<td>–</td>
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<tr>
<td>* (mean, range)</td>
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Table 2: Blocking autoantibodies against the folate receptor were found in serum specimens from 25 of 28 children with CFD, in 0 of 28 age-matched healthy controls, in 0 of 5 mothers of patients with CFD and in 0 of 41 patients with central nervous system disease unrelated to CFD [12]. The mean apparent $K_a$ for the binding of these autoantibodies to folate receptor was $5.54 \times 10^{10}$ litres per mole. (Reprinted with permission from [12]).
to the physiologically active 5MTHF [15, 16] enters the cerebrospinal fluid by way of the reduced folate carrier in the choroid epithelial cells. A second possibility is displacement of blocking autoantibodies to the folate receptors by a high level of 5MTHF (approximately 2 µM or greater). A third mechanism could be diffusion or another so far unknown transport mechanism, when the plasma level of 5MTHF is very high [12].

The origin of the blocking autoantibodies remains to be explained. Because of the appearance of the first clinical manifestations of cerebral folate deficiency after the age of four to six months the production of autoantibodies in these children probably occurred during the first four to six months of life. A transmission of autoantibodies from the mothers to their babies is less likely because the five mothers we tested had no autoantibodies. One possible induction of the autoantibody production could be contact with soluble folate-binding proteins in human or bovine milk or result from sensitization by unknown antigens with similar epitopes. Previous investigations could show that soluble-folate binding proteins in milk share amino acid sequence homology (91 percent similarity) with the membrane-bound folate receptors 1 and 2 that are expressed on human choroid plexus epithelium [17, 18]. Moreover, the human folate receptors on the choroid plexus cross-react with rabbit antibodies against the human-milk folate binding protein [19]. Autoantibodies against these epitopes could result in reduced folate transport into the cerebrospinal fluid.

The presence of blocking autoantibodies against the folate receptor at the choroid plexus in patients with CFD provides an autoimmune explanation for this hitherto underestimated and treatable condition. Early detection and diagnosis of cerebral folate deficiency are important because folinic acid at a pharmacologic dose can bypass autoantibody-blocked folate receptors, most likely by way of the reduced folate carrier. In this manner the folate pool within the central nervous system is restored. The clinical response in most patients is favourable, showing better social contact, motor improvements and reduction of seizures.

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REFERENCES:


**ADDRESS FOR CORRESPONDENCE:**

Vincent Thomas Ramaekers, MD PhD
Division of Paediatric Neurology
University Hospital Aachen
Pauwelsstrasse 30
D-52074 Aachen
Germany
E-Mail: vramaekers@ukaachen.de