Primary Necrotizing Lymphocytic Central Nervous System Vasculitis Due to Perforin Deficiency in a Four-Year-Old Girl

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We report the case of a 4-year-old girl who presented with headaches, ataxia, and visual disturbances. Cranial magnetic resonance imaging showed multiple supra- and infratentorial lesions with peripheral contrast enhancement and central necrosis. Brain biopsy revealed necrotizing lymphocytic vasculitis of undetermined etiology. Perforin expression was found to be significantly reduced in the patient's peripheral blood cells, and sequence analysis of the patient's perforin gene showed a compound heterozygous state with 1 nonsense mutation and 2 missense alterations in exon 2. Central nervous system (CNS) vasculitis was thus attributed to the perforin deficiency, and the patient was successfully treated by transplantation of stem cells from an HLA-identical brother. The findings described herein indicate that, even in the absence of classic non-neurologic symptoms of hemophagocytic lymphohistiocytosis, measurement of perforin expression should be one of the diagnostic tests used to identify the cause of unexplained CNS vasculitis, since this may have profound implications regarding therapy.

Primary central nervous system (CNS) vasculitis in children is a rare disease and is restricted to the vessels of the CNS, while secondary CNS vasculitis is

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associated with systemic conditions. Depending on the size, distribution, and degree of vascular stenosis, CNS vasculitis may lead to devastating neurologic deficits (1). Patients are often examined by rheumatologists because secondary CNS vasculitis can be part of systemic rheumatologic diseases (e.g., systemic lupus erythematosus [SLE] and antiphospholipid syndrome). Moreover, CNS inflammation is observed in hematologic diseases such as lymphoma or hemophagocytic lymphohistiocytosis (HLH).

Genetically determined forms of HLH include familial hemophagocytic lymphohistiocytosis (FHL), Chédiak-Higashi syndrome, Griscelli disease, and Purtilo syndrome, but HLH also may occur secondary to infection, tumors, metabolic disease (e.g., lysinuric protein intolerance), and autoimmunity (e.g., systemic juvenile rheumatoid arthritis). FHL is increasingly being characterized on a molecular genetic level. The first genetic cause identified for FHL was *PRF1* deficiency (2); recently, however, mutations in the hMunc13-4 and syntaxin 11 genes have been observed (3). The age at disease onset is usually <2 years.

FHL is a fatal disease, and the only curative therapy is hematopoietic stem cell transplantation (HSCT) (4). Diagnostic criteria for HLH were defined in 1991 by the Histiocyte Society and include fever; splenomegaly; cytopenia affecting ≥ 2 lineages; hypertriglyceridemia and/or hypofibrinogenemia; hemophagocytosis in bone marrow, spleen, or lymph nodes with no evidence of malignancy; low or absent natural killer (NK) cell cytotoxicity; hyperferritinemia; and elevated levels of soluble CD25 (4,5). Herein we report the case of a girl with perforin deficiency presenting as isolated CNS vasculitis in the absence of any of the classic symptoms of HLH.

CASE REPORT

The patient, a 4-year-old girl, was born by cesarean section in the 39th week of gestation as the first child

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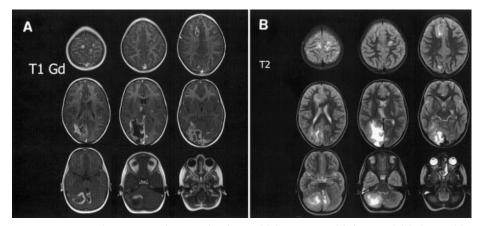


Figure 1. Magnetic resonance images, showing multiple supra- and infratentorial lesions, with peripheral contrast enhancement areas of central necrosis, and only minimal surrounding edema. **A**, T1-weighted images with intravenous administration of gadopentate dimeglumine. **B**, T2-weighted images.

of healthy, nonconsanguineous Russian parents of German descent. Her family medical history revealed vitiligo in her father and a healthy younger brother. She had an uneventful infancy and normal development until the age of 3 years 6 months, when she experienced visual disturbances and vomiting after physical efforts, sometimes associated with vertigo or dizziness.

Three months later, she presented with purpura 5 days after a slight upper respiratory tract infection with fever. Except for thrombocytopenia (<1,000 platelets/ μ l), laboratory results, including complete blood count, differential cell count, hemostasis, and levels of electrolytes, fibrinogen, transaminases, gamma glutamyl transferase, lactate dehydrogenase, and C-reactive protein (CRP), were normal. Bone marrow aspiration was not performed. Evaluation of the peripheral blood smear by an experienced hematologist revealed no abnormal cells. Idiopathic thrombocytopenic purpura (ITP) was diagnosed and the patient was treated with 2 mg/kg/day prednisolone for 3 days. Only after intravenous immunoglobulin was administered at a dose of 1 gm/kg/day on 2 consecutive days did a normalization of the platelet count occur. An electroencephalogram (EEG) was performed, and occipital deceleration was shown, predominantly on the right side. No cranial imaging was performed at that time.

At the age of 4 years 3 months, the patient was increasingly experiencing headaches and vomiting. In addition, she exhibited stumbling, falling, and loss of hearing. Physical examination revealed a slightly reduced muscular tonus, ataxia with a tendency to fall, and intentional tremor. There was no fever, hepatosplenomegaly, palpable peripheral adenopathies, or cutaneous or mucosal signs of purpura. Ophthalmologic examination did not show a papillar edema, but a scotoma was noted in the right eye. Findings on routine laboratory tests, including hematologic analysis, blood smear, and measurement of the erythrocyte sedimention rate and levels of CRP, IgG, IgA, IgM, electrolytes, fibrinogen, ferritin, and liver enzymes, were normal. Lumbar puncture showed a normal opening pressure, and analysis of the cerebrospinal fluid (CSF) showed no protein elevation, but a slight intrathecal production of antibodies was interpreted as a sign of a very discrete disruption of the blood-brain barrier. An EEG displayed intermittent deceleration on the right side, predominantly occipital. Cranial magnetic resonance imaging (MRI) showed multiple supra- and infratentorial lesions in both hemispheres (Figure 1).

Extensive microbiologic studies of the blood and CSF were performed. Blood serology showed positive IgG and negative IgM for measles, rubella, and mumps, indicating a normal response to vaccination, and clinically there had been no reactions to standard vaccination. Serology for human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV) revealed positive IgG levels, whereas IgM levels were negative. Polymerase chain reaction (PCR) analysis of CSF for many viruses, including HHV-6 and CMV, was negative. There was no evidence of active Epstein-Barr virus (EBV) infection (negative EBV-specific IgM by serologic testing and no detection of EBV by PCR in brain biopsy tissue). An extended survey for autoantibodies repeatedly revealed antinuclear antibody positivity (titer 1:160–1:320 by im-

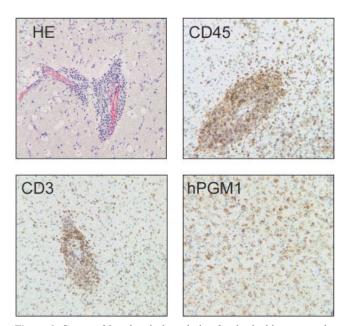


Figure 2. Immunohistochemical analysis of a brain biopsy sample. Sections were stained with hematoxylin and eosin (HE), anti-CD45 (leukocytes), anti-CD3 (T cells), and human phosphoglucomutase 1 (hPGM1) (macrophages). Diffuse, partly necrotizing lymphocytic vasculitis and diffuse meningoencephalitis involving a larger venous vessel and several smaller arteries are shown. (Original magnification \times 100.)

munofluorescence testing using HEp-2 cells, with a homogeneous or speckled pattern). Results of all other tests, notably those for DNase, antineutrophil cytoplasmic antibodies, anti–double-stranded DNA, and β_2 -glycoprotein antibodies, as well as anti–extractable nuclear antigen screening and complement analyses (CH50, AP50, C3, and C4), were normal.

The concern about starting immunosuppressive therapy before formally ruling out an acute infection and the diagnostic difficulty motivated us to perform a neurosurgical biopsy of the right occipital brain lesions. It showed T cell-mediated necrotizing lymphocytic vasculitis with no signs of demyelinization (Figure 2). Again, microbiologic culture and extended PCR-based analysis for infectious agents in the biopsy specimen did not reveal any microorganisms.

To treat the CNS vasculitis, methylprednisolone therapy (30 mg/kg/day for 5 days) was initiated and, in the second month of treatment, cyclophosphamide (500 mg/m² every 4 weeks) and prednisolone (2 mg/kg/day) were added. After 3 doses of cyclophosphamide at 500 mg/m², a control MRI of the brain showed slight progression of the lesions. Therefore, in month 5, the cyclophosphamide dose was increased to 1,000 mg/m² every 4 weeks. At that time, our attention was drawn to

a report by Feldmann and colleagues, describing patients with severe and progressive encephalitis as a presenting manifestation of a novel missense perforin mutation (6). Flow cytometry analysis of perforin expression on the patient's cells showed perforin to be almost absent on CD8+ cells and markedly reduced on CD56+ cells, even after in vitro stimulation of peripheral blood mononuclear cells (Figure 3).

These findings were consistent with perforin deficiency, which was confirmed by sequencing of the different exons of the gene coding for perforin in the patient. Several alterations were found in a heterozygous state in exon 2 of the patient's PRF1 gene: 1 nonsense mutation, Glu46stop, and 2 missense alterations, Ala91Val and Arg119Trp. The Glu46stop nonsense mutation in a heterozygous state was also found in the mother, whereas the 2 missense alterations were present in a heterozygous state in the father. Using a standard ⁵¹Cr release assay for mitogen- and interleukin-12-directed lysis, NK cell function was found to be significantly reduced (results not shown). Once the diagnosis of perforin deficiency was established, chemotherapy was initiated according to protocol HLH-94 of the Histiocyte Society (7). Severe neurologic complications occurred, including status epilepticus, necessitating intensive anticonvulsive therapy.

Perforin expression, NK cell function, and gene sequence were found to be normal in the patient's HLA-identical brother, so HSCT was performed 13

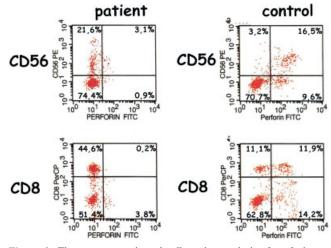


Figure 3. Fluorescence-activated cell sorting analysis of perform expression on cells from the patient and a healthy control. Perform expression was markedly reduced on the patient's CD56+ cells and almost absent on her CD8+ cells. PE = phycoerythrin; FITC = fluorescein isothiocyanate; PerCP = peridin chlorophyll protein.

months after presentation, after conditioning with fludarabine 30 mg/m²/day for 5 days starting 8 days prior to transplantation and with busulfan 4 mg/kg/day for 2 days starting 3 days before transplantation. HSCs were collected by cytapheresis. The patient received 3.5×10^6 CD34+ cells/kg body weight (8). Engraftment occurred with stable mixed chimerism, with ~20–30% persisting autologous cells in the peripheral blood and the bone marrow. Graft-versus-host disease prophylaxis was performed using cyclosporin A and mycophenolate mofetil from day 0 to day 28, with no signs of the disease.

Currently, the patient is doing well, with normal expression of perforin in the CD8+ cells (48% versus 51% in the control) and NK cells (70% versus 85% in the control) as well as normal NK cell function 5 months after bone marrow transplantation. Clinically, there has been a significant improvement in her language skills, neither ataxia nor seizures have been observed, and EEG results are nearly normal. Except for anticonvulsive therapy, the patient needs no more medication.

DISCUSSION

This case demonstrates that perforin deficiency may present as isolated vasculitis of the CNS. The differential diagnosis may include infection, malignancy, metabolic disease, and others, including demyelinating diseases. Although brain biopsy may be associated with local bleeding, edema, development of seizure focus, wound dehiscence, and infection, the overall incidence of brain biopsy-associated morbidity is low. Brain biopsy may thus be indicated in patients with unexplained CNS lesions to diagnose an underlying disease that is potentially treatable. In this case, the final diagnosis was made by histologic demonstration of nondemvelinating lymphocytic vasculitis, since MRI findings may mimic the findings observed in acute disseminating encephalomyelitis or multiple sclerosis (9). The differential diagnosis may also include SLE, because the patient's medical history included thrombocytopenia, ANA positivity, and CNS vasculitis. We believe SLE is highly unlikely in this case, however, since there were no cutaneous manifestations and no other organs were affected. Most important, anti-DNA antibodies were not detected and there was no hypocomplementemia. Further, increased, rather than decreased, perforin expression has been described in patients with SLE and in vasculitis syndromes (e.g., Behçet's syndrome, Takayasu arteritis) (10-12).

A surprising feature in this case was the late onset of disease and the absence of any classic associated non-neurologic HLH symptoms during the observation period. Recently, late disease onset (up to 12 years of age) was reported within a Japanese cohort of 40 FHL patients (13). In the patients described by Feldmann et al, the age at onset of neurologic signs was 5 months (death at 12 months) and 6 months (death at 44 months) (6). Parents were consanguineous, and both children developed the full clinical HLH manifestations to such an extent that stem cell transplantation could not be performed (6).

In retrospect, our patient's thrombocytopenia might be attributed to HLH. Because bone marrow aspiration was not performed, we cannot exclude the presence of hemophagocytosis at that time, despite the absence of any other HLH signs such as splenomegaly or laboratory changes. Slight neurologic alterations preceded the thrombocytopenia episode. The history was highly indicative of ITP, and response to intravenous immunoglobulin was observed. We favor the hypothesis that thrombocytopenia and the presence of ANAs in this case may be the result of a general immune dysregulation associated with perforin deficiency.

It has been hypothesized that the immune dysregulation in perforin deficiency may be triggered by childhood vaccinations or infections. In our case, it is interesting to note that, besides vaccination-induced immunity to measles, mumps, and rubella, the patient had positive IgG for CMV and HHV-6. The neurotropic HHV-6 and CMV can be reactivated and have repeatedly been associated with viral encephalitis in the immunocompromised host (14). The possibility cannot be excluded that in our patient, a previous infection with HHV-6 or CMV might have triggered the neurologic manifestations associated with the perforin defect. However, we were unable to detect any HHV-6 or CMV in the CSF or on brain biopsy.

The onset of the neurologic alteration was $\sim 6-9$ months before the first CNS imaging was performed. Reports in the literature have hinted at the diagnostic difficulty in recognizing FHL neurologic signs (15,16). Rooms et al reported 3 cases of HLH masquerading as child abuse (17). Two of the 3 children died from their disease shortly after presentation; the third survived, with a reported followup of several months after allogeneic bone marrow transplantation. Other observations concern 2 patients with acute encephalopathy as a primary manifestation of HLH, preceding, by 1 and 4 months, respectively, the classic laboratory findings (18), and 2 other children with progressive CNS disease as the main clinical manifestation, with HLH only being diagnosed postmortem after a review of the histopathologic material (19).

In conclusion, FHL must be considered an important differential diagnosis of isolated CNS vasculitis. even in the absence of any typical signs of HLH. A significant number of patients may not be correctly diagnosed or are never tested due to the lack of specific hallmarks. We suggest that FHL, on the basis of perforin deficiency, should be systematically included in the differential diagnosis of inflammatory diseases of the CNS in children. Testing for FHL can be simply performed by NK cell function assays and flow cytometry analysis of perforin expression. Gene sequencing may show missense mutations that abolish perforin function but not its expression on the cell surface (6). Once the formal diagnosis of FHL is achieved, allogeneic HSCT should be performed if possible, to avoid further complications and to reduce ultimate morbidity and mortalitv.

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AUTHOR CONTRIBUTIONS

Dr. Moshous had full access to all of the data in the study and takes responsibility for the integrity of the data.

Study design. Moshous, Niehues.

Acquisition of data. Moshous, Feyen, Lankisch, Schwarz, Schaper, Schneider, Schwahn.

Analysis and interpretation of data. Moshous, Feyen, Schaper, Niehues.

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Patient care during the bone marrow transplantation. Dilloo, Laws.

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