To the editor:

Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying PRF1 mutations

Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder of early infancy characterized by fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia, hypofibrinogenemia, and hemophagocytosis, which represent the diagnostic criteria. In the absence of a specific marker, differential diagnosis may be difficult, especially in patients without familial recurrence. Mutations in PRF1, a gene relevant for cellular cytotoxicity mechanisms whose mutations result in impaired antiviral defense and dysregulation of the apoptotic mechanisms, have been first documented in patients with HLH (HLH2; Online Mendelian Inheritance in Man #603553) by Stepp et al.3

The same clinical picture is apparently shared by patients with HLH due to PRF1 mutations or any other cause. Most patients develop HLH within the first few months of age. In the series of 122 patients collected by the International HLH Registry, most patients were diagnosed between 1 and 6 months. Exceptions to this general rule have been observed, as in the past there have been reports of familial cases with an age at onset of up to 8 years. Furthermore it has been observed that the age at onset for affected siblings is usually comparable and often coincides; discordant and later onset has been exceptionally reported. Clementi et al4 reported a PRF1 mutation in a 6-year-old patient, an age at which onset of HLH is rarely observed, and discussed whether adult forms of HLH2 may exist. We report 2 siblings sharing the same mutations in the PRF1 gene, who developed HLH at 22 and 21 years, respectively.

Case 1 is a white 27-year-old male, the son of unrelated parents from southern Italy. In 1996 at age 22, an occasional finding of hypertransaminasemia was reported, in the absence of any other clinical signs. A few months later, left arm weakness appeared, lasting one month. On July 1, 1998, he was admitted to the hospital because of spiking fever, cough, lymphadenopathy, pancytopenia, and neurologic symptoms (hemiplegia and somnolence). A brain magnetic resonance (MR) scan showed multifocal demyelination, but no clonal immunoglobulines were found in the cerebrospinal fluid (CSF). Neurosarcoidosis was suspected, and therapy with high doses of intravenous glucocorticoid was started, with good clinical response. Oral methylprednisolone was progressively tapered, but a minimal dose of 40 mg daily was required to control fever and asthenia; while pancytopenia persisted, the patient suffered recurrent respiratory and urinary infections, which required frequent hospitalizations.

We first saw the patient 2 years later, with fever, cough, dyspnea, herpes simplex lesions in nasal and perioral skin, marked hepatosplenomegaly, and severe cushingoid features. Laboratory findings showed pancytopenia, hypofibrinogenemia, hypertriglyceridemia, high levels of transaminases, colestatic indexes, and ferritin. Upper and lower respiratory infection by S aureus and P aeruginosa, together with septicemia by S hominis, were present. Re-evaluation of the bone marrow biopsy that had been performed elsewhere ruled out any malignancy; 8 months after this first one, a bone marrow biopsy was repeated with a liver biopsy, and both showed hemophagocytic features. Serologic tests for Epstein-Barr virus (EBV), cytomegalovirus (CMV), and adenovirus (IgM and IgG) were all negative, while a high IgG titer for herpes virus 1 and parvovirus B19 were found. In spite of the age of the patient, HLH was suspected. Natural killer (NK) cell activity was found completely absent, as was perforin expression by peripheral blood lymphocytes. Thus, starting in May 2000, the patient was treated with oral cyclosporin, with good clinical response. At the time of writing, he remains asymptomatic and laboratory values are normal except for hypertriglyceridemia.

Case 2 is the only sister of case 1 and is 25.5 years old at the time of writing. At 21 years, she was admitted to the hospital for spiking fever, weight loss, weakness, and hepatosplenomegaly. Pancytopenia, hypertriglyceridemia, hypofibrinogenemia and high levels of ferritin were observed. Neither superficial nor deep lymph nodes were enlarged, and an abdominal computed tomographic (CT) scan only showed moderate splenomegaly (19 cm) and mild hepatomegaly. Initial bone marrow biopsy showed a T-cell infiltrate with no evidence for clonal proliferation; this finding was confirmed at repeated biopsies. A T-cell lymphoblastic lymphoma was diagnosed, and the patient was treated with combination chemotherapy including vincristine, cyclophosphamide, asparaginase, doxorubicin, and intrathecal methotrexate; cranial irradiation was also given. One year later she underwent autologous bone marrow transplantation (BMT). In the follow-up mild cytopenia persisted for a few months. At the time of writing, 4 years after autologous BMT, she remains asymptomatic except for chronic fatigue. Laboratory tests only show mild leucopenia. When HLH was diagnosed in her brother, she also was tested for NK cell activity and perforin expression, both of which were found depleted.

Molecular analyses were performed as reported by Stepp et al,3 sequencing exons 2 and 3 of the PRF1 gene. The BLASTN program was used to compare the obtained sequences to the reported gene structure and to PRF1 genes of other species. The mutations found were tested for confirmation in the parents of the patients and in a group of 25 controls.

Sequencing of the coding exons of PRF1 disclosed the same mutations in both siblings: each of them carried both the Ala91Val and the Trp374Stop mutations (Figure 1). Each parent carried 1 of the 2 mutations in the heterozygous condition. The first mutation changes a small (alanine) into a larger (valine) hydrophobic amino acid and has not been reported previously. As the alanine at position 91 is conserved between human, mouse, and rat perforin and has not been found in either heterozygous or homozygous form in any other sequenced control (n = 31), it was inferred to be a pathogenic mutation. The second, truncating mutation was first reported by Stepp et al in families of Turkish origin and later found in patients from the same ethnic background by Goransdotter et al. In all cases it was present as homozygous mutation; it falls in an EGF domain, and the trypsin at position 374 is conserved between human, rat, and mouse perforin. It is a pathogenic mutation.

The presenting clinical picture of HLH is rather uniform, characterized by fever and hepatosplenomegaly with cytopenia and signs of hyperactivation and infiltration by lymphocytes and
macrophages, these last often engaged in hemophagocytosis. The central nervous system (CNS) is variably involved, with symptoms that range from irritability, bulging fontanel, and neck stiffness to seizures, cranial nerve palsies, ataxia, psychomotor retardation, and coma. Markedly different presenting pictures are not described in the available series.

Adult onset of familial HLH, never reported before, is the most prominent feature in this family. Their presenting features were different, with a prominent CNS involvement in one case and a diagnosis of non-Hodgkin lymphoma (NHL) in the other. Remarkably, in both cases the final diagnosis was delayed by about 3 years from the onset of symptoms; both achieved an adequate disease control when treated and did not show disease reactivation for more than one year after the diagnosis. Given the limited potential for antiviral defense in patients with PRF1 mutations, the prolonged absence of clinical manifestations due to macrophage activation following common viral infections in these subjects is unexpected. In case 2 the diagnosis of NHL led to polychemotherapy followed by autologous BMT. Her prolonged remission is also unexpected, as an autologous BMT consolidation has no potential to restore the genetically determined immune deficiency of the patient. We have previously observed in one HLH patient, after acute graft failure, an 18-month disease-free interval before overt disease reactivation (M.A. and F.L., unpublished observation, 1998). The mechanism by which massive immune suppression associated with the pre-BMT conditioning regimen may result in a prolonged disease control remains to be elucidated.

Even in the presence of quite different clinical presentations, both patients carried the same mutations. The Trp374Stop mutation has been previously reported in 9 patients always as a homozygous mutation. The presence of patients with atypical presentation makes the diagnosis of familial HLH, never reported before, the most prominent feature in this family. Their presenting features were different, with a prominent CNS involvement in one case and a diagnosis of non-Hodgkin lymphoma (NHL) in the other. Remarkably, in both cases the final diagnosis was delayed by about 3 years from the onset of symptoms; both achieved an adequate disease control when treated and did not show disease reactivation for more than one year after the diagnosis. Given the limited potential for antiviral defense in patients with PRF1 mutations, the prolonged absence of clinical manifestations due to macrophage activation following common viral infections in these subjects is unexpected. In case 2 the diagnosis of NHL led to polychemotherapy followed by autologous BMT. Her prolonged remission is also unexpected, as an autologous BMT consolidation has no potential to restore the genetically determined immune deficiency of the patient. We have previously observed in one HLH patient, after acute graft failure, an 18-month disease-free interval before overt disease reactivation (M.A. and F.L., unpublished observation, 1998). The mechanism by which massive immune suppression associated with the pre-BMT conditioning regimen may result in a prolonged disease control remains to be elucidated.

Even in the presence of quite different clinical presentations, both patients carried the same mutations. The Trp374Stop mutation has been previously reported in 9 patients always as a homozygous mutation. All these patients showed a homogeneous phenotype with a severe clinical course and an early age of onset, within the first months of life in 8 of 9 cases, with the ninth presenting at 39 months. This mutation was always found in subjects of Turkish origin; historical data are available that may support the hypothesis of migration of the mutation from Turkey to southern Italy in ancient times. Further research will clarify whether this mutation originated in the same haplotype both in the Italian and Turkish population.

We confirm that Trp374Stop appears to be the most frequently reported mutation in HLH and suggest that Ala91Val (which we found also in 4 other Italian patients; Clementi et al, in preparation) may be an “Italian mutation.” Thus the knowledge of the ethnic background of HLH patients may usefully address mutation analysis.

In our patients the genotype Trp374Stop/Ala91Val results in an atypical presentation and milder clinical course, which is possibly attributed to the latter mutation. Identification of patients with a milder clinical course and Ala91Val/Ala91Val genotype will confirm this hypothesis; whether they indeed share the usual HLH phenotype remains to be demonstrated.

The presence of patients with atypical presentation makes differential diagnosis in hemophagocytic syndromes more difficult. We suggest that HLH is included in the differential diagnosis of such patients not only in infants and children but also in adults. In the attempt to facilitate the diagnostic approach, we have recently proposed a flow chart, which includes testing for perforin expression, to improve the procedure.

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References


To the editor:

Transcription of AML1/ETO in bone marrow and cord blood of individuals without acute myelogenous leukemia

The translocation t(8;21), AML1/ETO, represents a frequent aberration in de novo acute myelogenous leukemia (AML) and is detectable in up to 40% of AML FAB M2. In constitutively transgenic mice, AML1/ETO abrogates fetal hematopoiesis, but in inducible transgenic mice AML1/ETO is not leukemogenic per se. AML1/ETO is detectable in stem cells of patients in complete continuous remission (CCR) and only an increasing transcript number indicates a forthcoming clinical relapse. We investigated...
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