Genetic predisposition to hemophagocytic lymphohistiocytosis: Report on 500 patients from the Italian registry

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare life-threatening disease affecting mostly children but also adults and characterized by hyperinflammatory features.
A subset of patients, referred to as having familial hemophagocytic lymphohistiocytosis (FHL), have various underlying genetic abnormalities, the frequencies of which have not been systematically determined previously.
Objective: This work aims to further our understanding of the pathogenic bases of this rare condition based on an analysis of our 25 years of experience.
Methods: From our registry, we have analyzed a total of 500 unselected patients with HLH.
Results: Biallelic pathogenic mutations defining FHL were found in 171 (34%) patients; the proportion of FHL was much higher (64%) in patients given a diagnosis during the first year of life. Taken together, mutations of the genes *PRF1* (FHL2) and *UNC13D* (FHL3) accounted for 70% of cases of FHL. Overall, a genetic diagnosis was possible in more than 90% of our patients with FHL. Perforin expression and the extent of degranulation have been more useful for diagnosing FHL than hemophagocytosis and the cytotoxicity assay. Of 281 (56%) patients classified as having "sporadic" HLH, 43 had monoallelic mutations in one of the FHL-defining genes. Given this gene dosage effect, FHL is not strictly recessive.
Conclusion: We suggest that the clinical syndrome HLH generally results from the combined effects of an exogenous trigger and genetic predisposition. Within this combination, different weights of exogenous and genetic factors account for the wide disease spectrum that ranges from HLH secondary to severe infection to FHL. (J Allergy Clin Immunol 2016;137:188-96.)

Key words: *Hemophagocytic lymphohistiocytosis, PRF1, UNC13D, immunologic tests*

In an article published in *The Lancet* in 1939, Bodley-Scott and Robb-Smith reported 4 patients originally given a diagnosis of "atypical Hodgkin disease."[1] The patients had unrelenting fever, lymphadenopathy, and rather massive hepatosplenomegaly. The authors stated that "where the cause of a morbid process is unknown its diagnosis commonly rests on the changes in the patient's tissues; thus ultimately the diagnosis can only be made with certainty by the microscope." In their cases the ultimate microscopic analysis was made postmortem because all 4 patients died. The authors were struck by the massive abundance of histiocytes in the spleen, liver, and other organs and by the prominent feature of erythrophagocytosis: they found 6 similar patients in the literature and coined the term "histiocytic medullary reticulosis."[1] Only the initial letter of histiocytes has survived in current acronyms, but we have little doubt that that article was the first clinicopathologic description of what we call today hemophagocytic lymphohistiocytosis (HLH). Some years later, Farquhar and Claireaux[2] described the first example of familial hemophagocytic lymphohistiocytosis (FHL), an entity for which other names in the literature have been "genetic" or "primary" HLH.[3,4] FHL is now defined on the basis of biallelic mutation in one of a set of functionally related genes or on familial recurrence. For clinical purposes, disease reactivation after initial remission has also been regarded as supporting the diagnosis of FHL.

From the functional point of view, in patients with FHL, defects of killing by T cells and natural killer (NK) cells have been prominent, and these can be identified by using appropriate tests. Over the last 15 years, it has been discovered that these defects are the consequence of mutations in one of a few genes (*PRF1*, *UNC13D*, *STX11*, and *STXBP2*) that are involved in exocytosis

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Abbreviations used
FHL: Familial hemophagocytic lymphohistiocytosis
HLH: Hemophagocytic lymphohistiocytosis
HSCT: Hematopoietic stem cell transplantation
MAS: Macrophage activation syndrome
NK: Natural killer
XLP: X-linked lymphoproliferative syndrome

of cytoplasmic granules and hence in perforin-mediated killing of target cells.[5-9] FHL can also be caused by mutations in other genes (*SH2D1A*, *XIAP*, *RAB27A*, *LYST*, and *AP3B1*) associated with the X-linked lymphoproliferative syndrome (XLP) disorders XLP1 and XLP2,[10] Griscelli syndrome type 2,[11] Chediak-Higashi syndrome,[12,13] and Hermansky-Pudlak syndrome.[14] The genetic defects in these patients (who sometimes have additional readily recognizable features) make them unable to effectively cope with the challenging pathogen, thus giving the picture of FHL.[15]

Cases with no evidence of familial recurrence have been called "secondary," but we prefer to call them "sporadic." For instance, HLH can develop as a complication of juvenile idiopathic arthritis (these cases have been called macrophage activation syndrome [MAS]) or during immunosuppressive therapies for cancer or autoimmune disorders,[4,16] although it can occur also in the course of protozoal (leishmaniasis and malaria), rickettsial,[17] or mycobacterial infection. In a proportion of cases of sporadic HLH, one finds cytocidal defects similar (although less profound) to those seen in patients with FHL. In the remaining cases of sporadic HLH, tests currently in use do not detect functional abnormalities, although we suspect that they exist.

The natural course of HLH is life-threatening (almost invariably so in patients with FHL) unless appropriate therapy is promptly instituted.[3] Two trials of the Histiocyte Society have established the combination of etoposide and dexamethasone as the standard of care.[18,19] This treatment is aimed to bridging the time gap until it is practical to carry out an allogeneic hematopoietic stem cell transplantation (HSCT), which, by replacing genetically defective bone marrow–derived cells with normal cells, provides cure for most patients.[20-22] Patients with sporadic disease have a similar clinical picture, and they respond to the etoposide/dexamethasone protocol as in patients with FHL. However, unlike patients with FHL, once in remission, they remain disease free, without any further therapy.[3,4,18,19] Thus patients with HLH are a dual diagnostic challenge. First, the clinical suspicion of HLH ought to be confirmed quickly, so that treatment can be started promptly to prevent organ damage and the risk of a fatal outcome. Second, it is important to distinguish FHL from sporadic HLH, so that HSCT can be organized speedily for patients with FHL and, just as importantly, patients with sporadic HLH are spared unnecessary HSCT.

Over the past 25 years, we have collected data on patients in whom a clinical diagnosis of HLH has been made by the attending clinicians. Here we report on those 500 patients in whom the diagnosis of HLH was subsequently confirmed.

METHODS

Starting from 1989, we centralized patient information and biologic samples to support the provisional clinical diagnosis of HLH and to perform immunologic and genetic studies of Italian patients, who were defined as such if living in Italy.[3] All centers of the national pediatric hematology-oncology cooperative group *(Associazione Italiana Ematologia Oncologia Pediatrica)* agreed to refer their patients' samples and information. Furthermore, in some cases samples of adult patients with a similar clinical picture are also referred.

HLH was defined according to the diagnostic criteria recommended by the Histiocyte Society (see Table E1 in this article's Online Repository at www.jacionline.org).[23] In 98 patients preliminarily reported as having HLH, the diagnosis was modified (acute infection, n = 19; cancer [lymphoma, n = 5; leukemia or myelodysplastic syndrome, n = 8; or brain tumor, n = 2], n = 15; liver failure, n = 12; encephalopathy, n = 12; autoimmune lymphoproliferative syndrome, n = 8; cytopenia, n = 8; other congenital disorder, n = 7; lysinuric protein intolerance, n = 5; primary immune deficiency, n = 4; congenital storage disease, n = 4; and Langerhans cell histiocytosis, inflammatory bowel disease, healthy subject, and other undefined, n = 1 each). Therefore these patients were excluded from this analysis.

Data on the family history and clinical and laboratory presenting features are collected on a specific form and stored in a dedicated Microsoft Access database. A unique patient number encodes the patients.

Peripheral blood samples from the patients are shipped overnight. Patients are tested before treatment start or during steroid monotherapy, which does not modify the results.[24] We perform immunologic screening assays,[25-30] and in case of abnormalities compatible with the clinical suspect, mutation analysis is started. In some patients receiving a diagnosis before immunologic assays were available or material from whom was inadequate for functional assays, mutation analysis was directly performed. The strategy of mutation analysis is as follows: in patients with defective perforin expression, *PRF1* is sequenced; in patients with a degranulation defect, *UNC13D*, *STX11*, and *STXBP2* are sequenced in that order; in patients with pigment deficiency, *RAB27a* and then *LYST* are sequenced; in male patients with defective SAP expression and/or inhibitory instead of activating 2B4 receptor function, *SH2D1A* is sequenced; and in male patients XIAP staining is also performed, and *XIAP* is sequenced. Archive cases with suspected but undefined genetic disease were progressively retrieved and analyzed when additional genes became available for testing.

Immunologic analyses and mutation analysis

Measurement of protein expression, granule release assays, cytotoxicity assays, and mutation analysis were performed, as previously described.[25-30] Details are available in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Statistical significance of the differences between the presence or absence of biallelic mutation for each age group has been evaluated by using the χ^2 test (GraphPad Prism 6; GraphPad Software, La Jolla, Calif). Differences have been considered significant at a P value of less than .05.

Ethics statement

The attending physician obtained written informed consent for data collection, immunologic studies, and genetic studies for all patients and family members. The study was approved by the Institutional Review Board of the A.O.U. Meyer, Florence, Italy. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

RESULTS

Study population

The mean annual accrual to the Italian Registry increased steadily over the study period (see Table E2 in this article's Online Repository at www.jacionline.org), with a total of 500 patients enrolled. There were 259 male and 241 female patients. Age at the time of diagnosis ranged from 0 to 60 years (Fig 1), with a median of 2.2 years. Although the vast majority of patients were children, 44 (8.8%) were older than 18 years at the time of diagnosis.