Autoimmune Lymphoproliferative Syndrome Misdiagnosed as Hemophagocytic Lymphohistiocytosis

abstract

Autoimmune lymphoproliferative syndrome (ALPS) is a rare inherited disorder of apoptosis, most commonly due to mutations in the *FAS* (*TNFRSF6*) gene. It presents with chronic lymphadenopathy, splenomegaly, and symptomatic multilineage cytopenias in an otherwise healthy child. Unfortunately, these clinical findings are also noted in other childhood lymphoproliferative conditions, such as leukemia, lymphoma, and hemophagocytic lymphohistiocytosis, which can confound the diagnosis. This report describes a 6-year-old girl with symptoms misdiagnosed as hemophagocytic lymphohistiocytosis and treated with chemotherapy before the recognition that her symptoms and laboratory values were consistent with a somatic *FAS* mutation leading to ALPS. This case should alert pediatricians to include ALPS in the differential diagnosis of a child with lymphadenopathy, splenomegaly, and cytopenias; obtain discriminating screening laboratory biomarkers, such as serum vitamin B-12 and ferritin levels; and, in the setting of a highly suspicious clinical scenario for ALPS, pursue testing for somatic *FAS* mutations when germ-line mutation testing is negative. *Pediatrics* 2013;132:e1440–e1444

AUTHORS: Amanda Rudman Spergel, MD,^a Kelly Walkovich, MD,^b Susan Price, RN,^a Julie E. Niemela, MS,^c Dowain Wright, MD, PhD,^d Thomas A. Fleisher, MD,^c and V. Koneti Rao, MD^a

^aALPS Unit, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, and ^cDepartment of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland; ^bDivision of Pediatric Hematology/Oncology, University of Michigan, Ann Arbor, Michigan; and ^dDivision of Rheumatology and Immunology, Children's Hospital Central California, Madera, California

KEY WORDS

cytopenias, splenomegaly, lymphadenopathy, HLH, ALPS, apoptosis

ABBREVIATIONS

ALPS—autoimmune lymphoproliferative syndrome

ALPS-FAS—autoimmune lymphoproliferative syndrome due to germ-line *FAS* mutations

ALPS-sFAS—autoimmune lymphoproliferative syndrome due to somatic *FAS* mutations

DNT—double-negative T cell

HLH—hemophagocytic lymphohistiocytosis

IL—interleukin

sIL-2R α —soluble interleukin 2 receptor α

All authors made substantial contributions to the conception and design, acquisition of data, and analysis and interpretation of data as outlined below. Dr Rudman Spergel evaluated the patient under discussion, helped in acquisition, analysis, and interpretation of data, drafted the initial manuscript, and reviewed and revised the manuscript; Dr Walkovich assisted with review of data and writing of the manuscript after reviewers' comments; Ms Price coordinated and supervised data collection for the cohort of patients, conducted initial analysis of data from the entire cohort, and critically reviewed the manuscript; Ms Niemela provided laboratory support for sequencing the *FAS* gene and interpretation of the mutation data across the cohort and critically reviewed the manuscript; Dr Wright suspected the diagnosis and referred the patient to the National Institutes of Health (NIH) for further workup, provided patient care, helped in revising the manuscript critically for important intellectual content, and critically reviewed the manuscript; Dr Fleisher provided supervision and clinical immunology laboratory support for the diagnostic workup of all patients with autoimmune lymphoproliferative syndrome (ALPS) at the NIH and critically reviewed the manuscript; and Dr Rao and his team provided diagnosis and patient care at the NIH ALPS clinic, conceptualized and initiated the drafting of this manuscript as a case report, and helped with critical revisions of the manuscript through many versions for intellectual content; and all authors approved the final manuscript as submitted.

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First recognized in the early 1990s,¹ autoimmune lymphoproliferative syndrome (ALPS) is a rare inherited disorder of apoptosis leading to autoimmune cytopenias due to immune dysregulation and inappropriate accumulation of lymphocytes in the lymph nodes and spleen. Because the differential diagnosis for lymphadenopathy, splenomegaly, and cytopenias involves many conditions with overlapping features, diagnostic criteria for ALPS (Table 1) and other lymphoproliferative disorders, such as hemophagocytic lymphohistiocytosis (HLH) (Table 2), have been developed.^{2,3} By definition, ALPS patients have chronic nonmalignant lymphadenopathy and/or splenomegaly associated with an increased circulating population of pathognomonic CD3⁺ TCR $\alpha\beta$ ⁺ lymphocytes that do not express CD4 or CD8, which are referred to as double-negative T cells (DNTs). In addition, patients frequently have classically elevated biomarkers (eg, vitamin B-12). The most common cause of ALPS is an inherited germ-line heterozygous mutation in the *FAS* (*TNFRSF6*) gene, known as ALPS-FAS. Recently, an increasing cohort of patients with somatic *FAS* gene mutations that are mostly limited to the circulating DNTs (ALPS-sFAS) have been described with the same clinical and laboratory features as patients with germ-line mutations^{4,5}. In this case report, we describe a 6-year-old girl with ALPS-sFAS after a presumptive diagnosis of HLH was made in her first year of life.

PATIENT PRESENTATION

The patient was born at full term and was clinically well during the first months of life. However, routine laboratory testing at her 9-month well-child visit revealed pancytopenia (white blood cell count: 4700 cells per mm³; absolute neutrophil count (ANC): 1200 per mm³; hemoglobin: 6.3 g/dL; platelets: 87 000 per mm³), prompting a subspecialty referral and

TABLE 1 Revised Diagnostic Criteria for ALPS According to the First International ALPS Workshop 2009

Criteria to Determine a Diagnosis of ALPS
Required criteria
Chronic (>6 mo), nonmalignant, noninfectious lymphadenopathy and/or splenomegaly
Elevated CD3 ⁺ TCR $\alpha\beta$ ⁺ CD4 ⁻ CD8 ⁻ DNTs (>1.5% of total lymphocytes or >2.5% of CD3 ⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts
Accessory criteria
Primary
Defective lymphocyte apoptosis
Somatic or germ-line pathogenic mutation in <i>FAS</i> , <i>FASLG</i> , or <i>CASP10</i>
Secondary
Elevated plasma sFASL levels (>200 pg/mL), plasma IL-10 levels (>20 pg/mL)
Serum or plasma vitamin B-12 levels (>1500 pg/mL) or plasma IL-18 levels >500 pg/mL
Typical immunohistologic findings
Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia)
Elevated IgG levels (polyclonal hypergammaglobulinemia)
Family history of a nonmalignant/noninfectious lymphoproliferation with or without autoimmunity
Definitive diagnosis: both required criteria plus 1 primary accessory criterion
Probable diagnosis: both required criteria plus 1 secondary accessory criterion

Reprinted with permission from Oliveira JB, Bleesing JJ, Dianzani U, et al. *Blood*. 2010;116(14):e35–e40. IgG, immunoglobulin G; sFASL, Soluble FasLigand.

TABLE 2 Diagnostic Criteria for HLH Used in the HLH-2004 Trial

Criteria to Determine a Diagnosis of HLH ^a
(A) A molecular diagnosis consistent with HLH: pathologic mutations of <i>PRF1</i> , <i>UNC13D</i> , <i>Munc18-2</i> , <i>Rab27a</i> , <i>STX11</i> , <i>SH2D1A</i> , or <i>BIRC4</i>
Or
(B) If 5 of the 8 criteria listed below are fulfilled:
1. Fever $\geq 38.5^{\circ}\text{C}$
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)
Hemoglobin <9 g/dL (in infants <4 weeks: hemoglobin <0 g/dL)
Platelets <100 $\times 10^3$ per mL
Neutrophils <1 $\times 10^3$ per mL
4. Hypertriglyceridemia (fasting: >265 mg/dL) and/or hypofibrinogenemia (<150 mg/dL)
5. Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver
6. Low or absent NK-cell activity
7. Ferritin >500 ng/mL ^b
8. Elevated sCD25 (α -chain of sIL-2 receptor) ^c

Reprinted with permission from Jordan MB, Allen CE, Weitzman Sheila, et al. *Blood*. 2011;118:4041–4052. NK, natural killer; sCD25, Soluble CD25 (a term sometimes used for α -chain of soluble IL-2 receptor).

^a In addition, in the case of familial HLH, no evidence of malignancy should be apparent.

^b Although the HLH-2004 protocol uses ferritin >500 ng/mL, we generally view ferritin levels >3000 ng/mL as concerning for HLH and ferritin levels >10 000 as highly suspicious.

^c Elevations above age-adjusted, laboratory-specific normal levels (defined as >2 SDs from the mean) appear to be more meaningful than the original designation of >2400 U/mL because of variations between laboratories.

a hospital admission. Her spleen was palpable 5 cm below the left costal margin, her liver edge was palpable 4 cm below the right costal margin, and she had palpable cervical lymphadenopathy. After viral etiologies were ruled out, a diagnosis of HLH was considered because she met 4 of 8 criteria including the following: cytopenias involving at least 2 cell lineages, hypertriglyceridemia (292 mg/dL), increased

soluble interleukin (IL) 2 receptor α (sIL-2R α) level (18651 U/mL), and splenomegaly. Notably, she did not have persistent fevers. She also had normal fibrinogen and ferritin levels, as well as normal natural killer cell activity. On biopsy, her bone marrow and lymph node tissues showed no evidence of hemophagocytosis. A repeat ferritin measurement 1 month later was 809 ng/mL, providing a fifth criterion for

HLH. Genetic evaluation for familial HLH revealed a variant of uncertain significance in the gene encoding perforin (c.272C>T, p.A91V). Treatment of HLH was initiated per the HLH-2004 Therapeutic Guidelines for Hemophagocytic Lymphohistiocytosis, consisting of etoposide, dexamethasone, and cyclosporine due to persistent and worsening lymphoproliferation and transfusion-dependent cytopenias.⁶ Because she remained treatment refractory, a search for a matched unrelated donor for allogeneic bone marrow transplantation was initiated, but transplant efforts were significantly delayed due to her complex social situation. Chemotherapy was stopped in October 2008 after 22 consecutive months of treatment. To obtain a more definitive tissue diagnosis and because the patient was experiencing discomfort from her significantly enlarged spleen, a splenectomy was performed in January 2009, both for therapeutic and diagnostic purposes. The pathology report after splenectomy revealed an enlarged spleen showing

hypersplenism with widened splenic cords and rare to absent hemophagocytic activity. A second opinion noted that the spleen also had atypical T-cell hyperplasia with DNTs, consistent with ALPS. Mutation analyses for the ALPS-related genes (*FAS*, *FASL*, *CASP10*) were then obtained; however, no mutations were detected in genomic DNA obtained from peripheral blood mononuclear cells. Other screening tests for ALPS (Table 1), including serum vitamin B-12 levels, DNT percentage, and apoptosis assays, were not obtained. She was subsequently referred to the National Institutes of Health for further workup to evaluate for somatic ALPS. Peripheral blood immunophenotyping revealed an increased percentage of circulating DNTs (25.9%). In addition, she was determined to have an elevated serum vitamin B-12 level (>6000 pg/mL), high serum IL-10 (592 pg/mL), and high soluble FASL (5448 pg/mL). These serum biomarkers together with the elevated DNTs supported the diagnosis of ALPS caused by a *FAS* mutation.⁷ A diagnosis of ALPS-sFAS was confirmed

by detecting a somatic *FAS* mutation (c.913delTinsGA, p.M224RfsX7) in genomic DNA extracted from isolated DNTs.

DISCUSSION

ALPS and HLH have many overlapping clinical and laboratory features. Both are lymphoproliferative syndromes that present in childhood and are characterized by persistent lymphadenopathy/splenomegaly with evidence of immune dysregulation that includes hyperinflammation and cytopenias. Although underappreciated, ALPS-FAS and ALPS-sFAS patients often have significantly elevated sIL-2R α and can occasionally have evidence of hemophagocytes in the bone marrow, making the distinction between ALPS and HLH even more nebulous. However, distinguishing between ALPS and HLH is crucial because the management paradigms are very different. ALPS patients often require long-term immunosuppressive therapies with corticosteroids and steroid-sparing measures, including mycophenolate mofetil

FIGURE 1 Biomarkers in patients with ALPS-FAS and ALPS-sFAS. A, Data collected from 142 unique ALPS-FAS/ALPS-sFAS patients aged ≤ 26 years old were used to generate box plots for ferritin, sIL-2R α , and vitamin B-12 values. Boxes represent the 25th–75th percentile of values; the median is indicated by a solid dark line within each box; whiskers represent the 2.5th–97.5th percentile of values; and outlier data points are shown as dark circles. B, Specific numerical data for the median, 25th percentile, 75th percentile, minimum-maximum range, and number of observations for each biomarker. Not all patients had all biomarker values available for this analysis. Within-person median values were analyzed when > 1 data point was available for any individual. Vitamin B-12 levels were capped at an upper range of 4000 pg/mL because serial dilutions of plasma in our laboratory had shown vitamin B-12 values as high as 40 000 pg/mL in several patients with ALPS-FAS. Max, maximum; Min, minimum.

or sirolimus for managing chronic, refractory cytopenias and hypersplenism, respectively.⁸ ALPS patients with germ-line *FAS* mutations also require regular vigilance for possible hematopoietic malignancies because their risk of developing Hodgkin and non-Hodgkin lymphoma is 50- and 14-fold greater, respectively, than in the general population.⁹ Although splenectomy provided the tissue diagnosis of ALPS in this patient, it is neither necessary nor desirable for the diagnosis and management of most patients with ALPS.^{8,10,11} In contrast, the current standard of care is to treat HLH patients with chemotherapy, including etoposide and dexamethasone plus or minus cyclosporine. If the patient has a family history of HLH, has one of the genetic mutations, relapses while on chemotherapy for HLH, and/or has central nervous system disease, allogeneic bone marrow transplantation is required for an effective cure.³

In this case, the initial presumptive diagnosis of HLH delayed the identification of her actual diagnosis for almost 2 years. In the interim, she was exposed to 22 months of chemotherapy and underwent splenectomy before a definitive diagnosis of ALPS-sFAS was established. Although, she currently requires no specific treatment of her ALPS, there is now a need to carefully monitor her long term for risk of pneumococcal sepsis due to her surgical asplenia and the possibility of secondary leukemia associated with exposure to etoposide therapy.¹² Both of these risks could have been avoided had the diagnosis of

ALPS been considered and recognized sooner.

To distinguish the 2 disorders, it is essential to obtain discriminating data early as indicated in the diagnostic criteria for ALPS (Table 1) and HLH (Table 2). Elevated serum biomarkers (vitamin B-12, sFASL, IL-10, IL-18) in combination with evidence of autoimmune cytopenias and elevated immunoglobulin G levels can point toward a diagnosis of ALPS. However, recent reports also note elevated IL-10 levels in some HLH patients.^{13,14} In the case of germ-line mutations, a family history of chronic lymphoproliferation can support the diagnosis of ALPS. Importantly, the ferritin level can help differentiate ALPS from HLH, because the ferritin elevation in ALPS patients is generally lower than 3000 ng/mL, which is reportedly more specific for HLH.¹⁵ In our cohort of patients with ALPS-FAS and ALPS-sFAS who were ≤ 26 years old, only 1 patient, who notably had received numerous blood transfusions, had a ferritin level > 3000 ng/mL. The remaining patients had serum ferritin levels ranging from 18 to 2951 ng/mL (median: 138 ng/mL), with 67 out of the 78 samples tested for ferritin found to be < 500 ng/mL. Patient samples were also remarkable for very high median sIL-2R α (5645 units/mL) and serum vitamin B-12 (3444 pg/mL) levels (Fig 1). We have highlighted this case to emphasize the importance of considering rare disorders, particularly ALPS, in the differential diagnosis of patients presenting with lymphadenopathy, spleno-

megaly, and cytopenias. Additionally, simple biomarkers should always be sought early in evaluation before obtaining more expensive, and potentially confounding, genetic testing. Readily attainable ALPS-related biomarkers (Table 1, Fig 1) can be used to determine the likelihood of ALPS and the need for sending confirmatory genetic testing. Last, it is critical to recognize that the clinical features of ALPS-FAS/ALPS-sFAS are similar, but Sanger sequencing using genomic DNA from unseparated peripheral blood mononuclear cells can lead to false-negative results in ALPS-sFAS. When there is a strong clinical likelihood of ALPS, but mutation analysis using genomic DNA has failed to reveal a *FAS* mutation, the analysis should be repeated by using DNA extracted from isolated peripheral blood DNTs to establish a diagnosis of ALPS-sFAS.⁵

ACKNOWLEDGMENTS

The authors thank Dr Kenneth McClain, Director, Histiocytosis Program at Texas Children's Cancer and Hematology Center, and Dr Kelly Stone, Director, Allergy Immunology Fellowship Program, NIAID, NIH, for reviewing the manuscript and for providing valuable comments, and Ms Katie Perkins and Dr Ronald Hornung for providing critical support related to clinical care and laboratory assays. They also gratefully acknowledge the invaluable contribution and generous help from the NIAID biostatistician, Dr Pamela Shaw, with data analysis to generate Fig 1.

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This study has undergone annual review by the NIAID Institutional Review Board, and all participants whose data are included in this article were enrolled in the study after undergoing the informed consent process. This manuscript was developed after a presentation and discussion of this patient at the weekly grand rounds of the NIAID, NIH, in Bethesda, Maryland, in 2012.

This trial has been registered at www.clinicaltrials.gov (identifier: NCT00001350).

www.pediatrics.org/cgi/doi/10.1542/peds.2012-2748

doi:10.1542/peds.2012-2748

Accepted for publication Jun 5, 2013

Address correspondence to V. Koneti Rao, MD, ALPS Unit, LI/NIAID, National Institutes of Health, DHHS, Room 12C106, Building 10, 10 Center Dr, Bethesda, MD 20892-1899. E-mail: krao@niaid.nih.gov

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: The Intramural Research Program of the National Institute of Allergy and Infectious Diseases and the National Institutes of Health Clinical Center supported this research. Funded by the National Institutes of Health (NIH).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.