Understanding human immunology through the study of primary immune deficiency disorders

de Oliveira Filho, J.B.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)

Download date: 30 Mar 2019
CHAPTER 2

REVISED DIAGNOSTIC CRITERIA AND CLASSIFICATION FOR THE AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS): REPORT FROM THE 2009 NIH INTERNATIONAL WORKSHOP.

Oliveira JB1, Bleesing JJ2, Dianzani U3, Fleisher TA1, Jaffe ES4, Lenardo MJ5, Rieux-Laucat F6, Siegel RM7, Su HC8, Teachey DT9, Rao VK10

1 Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, USA; 2 Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, USA; 3 Interdisciplinary Research Centre of Autoimmune Diseases, and University of Eastern Piedmont, Novara, Italy; 4 Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; 5 Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; 6 INSERM U768, Université Paris Descartes, Paris, France; 7 Immunoregulation Unit, Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD; 8 Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA; 9 Division of Hematology and Oncology, Department of Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, PA, USA; 10 ALPS Unit, Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

ABSTRACT
Lymphadenopathy in children for which no infectious or malignant etiology can be ascertained constitutes a challenging diagnostic dilemma. Autoimmune lymphoproliferative syndrome (ALPS) is a human genetic disorder of lymphocyte apoptosis resulting in an accumulation of lymphocytes and childhood onset chronic lymphadenopathy, splenomegaly, multilineage cytopenias, and an increased risk of B-cell lymphoma. In 1999, investigators at the National Institutes of Health (NIH) suggested criteria to establish the diagnosis of ALPS. Since then, with approximately 500 ALPS patients studied worldwide, significant advances in our understanding of the disease have prompted the need for revisions to the existing diagnostic criteria and classification scheme. The rationale and recommendations outlined here stem from an international workshop held at NIH on September 21-22, 2009, attended by investigators from the USA, Europe and Australia engaged in clinical and basic science research on ALPS and related disorders. It is hoped that harmonizing the diagnosis and classification of ALPS will foster collaborative research and better understanding of the pathogenesis of autoimmune cytopenias and B cell lymphomas.

INTRODUCTION
Lymphadenopathy in children with no known infectious or malignant etiology constitutes a challenging diagnostic dilemma. A recently described entity that defines some children with previously unexplained lymphadenopathy is the autoimmune lymphoproliferative syndrome (ALPS)\textsuperscript{1,2}. The clinical antecedents to ALPS entail various syndromes of familial chronic nonmalignant lymphadenopathy and splenomegaly including pseudomononucleosis, pseudolymphoma, and the Canale-Smith syndrome\textsuperscript{3,4,5}. In 1992, Sneller and co-workers recognized that these entities resembled two related mouse strains with lymphoproliferative phenotypes known as \textit{lpr} (lymphoproliferation) and \textit{gld} (generalized lymphoproliferative disease)\textsuperscript{6}. Earlier that same year the molecular defect of the \textit{lpr} mouse was shown to be a loss of function mutation in a “death receptor” gene that is a member of the tumor necrosis factor receptor (TNFR) superfamily, \textit{FAS/CD95/APO-}
1/TNFRSF6\(^7\). Subsequently, this association was validated in humans when the underlying defect in two series of patients with a lymphoproliferative disorder was determined to be a failure of lymphocyte apoptosis due to a mutation in FAS\(^{4,2}\). The mutations resulted in the accumulation of proliferating lymphocytes with childhood onset chronic lymphadenopathy, splenomegaly, multilineage cytopenias secondary to sequestration and autoimmune destruction, and an increased risk of B-cell lymphoma\(^{6,8,9}\). Laboratory findings included polyclonal hypergammaglobulinemia and expansion of a unique population of circulating TCR\(^{\alpha\beta}\)B220\(^+\)CD4\(^-\)CD8\(^-\) T lymphocytes, referred to as TCR\(^{\alpha\beta}\) double negative T (TCR\(^{\alpha\beta}\) -DNT) cells throughout this paper\(^8,9\).

The majority of ALPS patients harbor heterozygous germline mutations in FAS, inherited in an autosomal dominant fashion\(^{10,11}\). Interestingly, somatic FAS mutations are the second most common genetic etiology of ALPS\(^{12,13}\). Additionally, mutations in the genes encoding FAS ligand, caspase 10, caspase 8, and NRAS have been identified in a minority of patients with ALPS and related disorders\(^{14-19}\). Approximately one third of ALPS patients have yet unidentified genetic defects.

In 1999, investigators at the National Institutes of Health (NIH) suggested a triad of criteria to establish the diagnosis of ALPS (Table 1)\(^{20,21}\). Since then, important advances have been made in our understanding of the disease. Here we would like to propose several revisions to the current diagnostic criteria and classification system. The recommendations stem from a workshop held at the NIH in the fall of 2009 attended by investigators from the USA, Europe and Australia engaged in clinical and basic science research pertaining to ALPS and related disorders. The changes proposed follow the deliberations at the meeting leading to a consensus after further teleconferences and electronic communications among the coauthors of this document. It is hoped that these modifications will harmonize and simplify the diagnosis and classification of ALPS, facilitating collaboration and data exchange between different clinicians and research centers across the globe. A scientific summary of the meeting proceedings has been published elsewhere\(^{22}\).
Table 1. Diagnostic criteria for ALPS as defined in 1999

<table>
<thead>
<tr>
<th>Required criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Chronic non-malignant lymphadenopathy and/or splenomegaly</td>
</tr>
<tr>
<td>- Increased peripheral CD3⁺ TCRαβ⁺CD4⁻CD8⁻ (DNT) cells</td>
</tr>
<tr>
<td>- Lymphocyte apoptosis defect</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Supporting criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Family history of ALPS</td>
</tr>
<tr>
<td>- Characteristic histopathology</td>
</tr>
<tr>
<td>- Autoimmune manifestations</td>
</tr>
</tbody>
</table>

MODIFICATIONS TO THE DIAGNOSTIC CRITERIA OF ALPS

Rationale

Reevaluation of the currently used ALPS diagnostic criteria (Table 1) suggested several potential problems hindering its widespread use:

1. The lymphocyte apoptosis assay, currently an absolute requirement for diagnosis, is resource-intensive to perform, available only in selected centers, and may be unable to identify patients with somatic FAS or germline FASLG mutations; moreover, methodology is not standardized among centers, often leading to variable results;
2. The current definition does not incorporate genetic information or other biomarkers that have recently been shown to predict ALPS;
3. Evaluation of a large number of control samples in different centers suggests that a diagnostic cutoff for TCRαβ⁺DNT cells of 1% of total lymphocytes does not always accurately predict ALPS;
4. Histopathological findings, highly characteristic of ALPS in some cases, and compatible family history are not currently utilized for diagnosis.

Recommendations

This revision divides diagnostic criteria for ALPS into two required and six accessory criteria (Table 2). Required criteria include the presence of lymphadenopathy and/or splenomegaly, and elevated TCRαβ⁺-DNT cells. Accessory criteria are subdivided into
Revised diagnostic criteria and classification of ALPS

primary, which include an abnormal lymphocyte apoptosis assay and the presence of pathogenic mutations in genes of the FAS pathway; and secondary, which include the presence of elevated circulating biomarkers, characteristic histopathology, the combined presence of autoimmune cytopenias, polyclonal hypergammaglobulinemia, and family history compatible with ALPS. These criteria are discussed in detail below.

For a definitive ALPS diagnosis a patient has to meet both required criteria and one of the primary accessory criteria (Table 2). A probable ALPS diagnosis can be entertained by the presence of the required criteria and any one of the secondary accessory criteria. From a clinical perspective, patients with probable ALPS should be treated and monitored in the same way as patients with a definitive diagnosis, but physicians are advised to pursue a genetic or apoptosis assay based diagnostic work up whenever possible.

There is an absolute requirement for the presence of lymphadenopathy and/or splenomegaly persistent for more than 6 months. If isolated, the lymphadenopathy has to affect at least two nodal chains. Neoplastic and infectious etiologies must be excluded. In many cases associated hepatomegaly may also be present, but in isolation it is not a diagnostic criterion. The lymphadenopathy in ALPS typically fluctuates and involves the cervical, axillary and inguinal chains, although mesenteric, retroperitoneal, pelvic, and mediastinal lymph node expansions are also often noted by imaging studies.

The second required ALPS criterion is the presence of elevated circulating TCRαβ⁺-DNT cells, a hallmark of this disease. This population must be clearly distinguished from TCRγδ⁺DNT cells by co-staining with TCRαβ⁺ directed antibodies. Rare conditions unrelated to ALPS may present with an expansion of Natural Killer T (NKT) cells, which can be CD3⁺ TCRαβ⁺CD4⁻CD8⁻, and these can be distinguished from ALPS specific DNTs by co-staining with NKT markers such as CD16, CD56, Vα24Vβ11 or α-GalCer-CD1d tetramers; however, we do not recommend such extended staining routinely in ALPS investigation. The staining protocol currently used by the NIH Immunology Service can be found in Supplementary File 1.
Table 2. Revised Diagnostic Criteria for ALPS

**REQUIRED**

1. Chronic (>6 months), non-malignant, non-infectious lymphadenopathy and/or splenomegaly.
2. Elevated CD3⁺ TCRαβ⁺CD4⁻CD8⁻ DNT cells (equal to or greater than 1.5% of total lymphocytes or 2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts.

**ACCESSORY**

*Primary*

1. Defective lymphocyte apoptosis (in 2 separate assays);
2. Somatic or germline pathogenic mutation in FAS, FASLG, or CASP10;

*Secondary*

1. Elevated plasma sFASL levels (>200 pg/ml) OR elevated plasma IL-10 levels (>20 pg/ml) OR elevated serum or plasma Vitamin B12 levels (>1500 ng/L) OR elevated plasma IL-18 levels above 500 pg/ml;
2. Typical immuno-histological findings as reviewed by an experienced hematopathologist;
3. Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated IgG levels (polyclonal hypergammaglobulinemia);
4. Family history of a non-malignant/non-infectious lymphoproliferation with or without autoimmunity.

**Definitive diagnosis:** Both required criteria plus one primary accessory criterion.

**Probable diagnosis:** Both required criteria plus one secondary accessory criterion.

A review of data, including pediatric controls, gathered from different centers showed that TCRαβ⁺-DNT levels between 1.0 and 1.5% of total lymphocytes may be observed in normal individuals or as a reactive phenomenon in conditions such as systemic lupus erythematosus (SLE), and hence their presence as an isolated finding should not prompt screening for ALPS\(^{24-26}\) (and Bleesing JJ, personal communication). As a consequence of these observations, the percentage of TCRαβ⁺-DNT cells required for a diagnosis has been revised to be greater than or equal to 1.5% of total lymphocytes (or 2.5% of T lymphocytes), in the setting of normal or elevated lymphocyte counts. The presence of lymphopenia invalidates this criterion, as its impact on the relative distribution of TCRαβ⁺-DNT cells is unknown. Absolute counts of TCRαβ⁺-DNT cells
Revised diagnostic criteria and classification of ALPS

will vary by age. Elevations of TCRαβ⁺DNT cells above 3% of the total lymphocytes (or >5% of T lymphocyte cells) are rarely, if ever, seen in conditions other than ALPS and are therefore essentially pathognomonic for this disease. Each testing laboratory should ideally develop its own reference ranges, adjusted for age, and provide both percent and absolute numbers of this lymphocyte subset in the patient report.

Primary accessory criteria include an abnormal lymphocyte apoptosis assay (Table 2). This test is no longer considered essential for the diagnosis of ALPS, as patients with somatic FAS mutations and germline FASLG mutations typically are found to have normal FAS-induced apoptosis assays. However, the presence of a reproducible apoptotic defect in patients who fulfill the Required criteria is enough for a diagnosis of ALPS. Given the high inter-laboratory variability in the protocol used for this assay, a repeat assay for confirmation is now required. Acceptable apoptosis tests in cultures of activated primary T cells include direct FAS activation using cross-linked agonistic antibodies or recombinant Fas ligand or TCR re-stimulation. Detailed description of the protocols utilized for apoptosis detection and measurement can be found in recent related publications. Patient results must be compared to normal controls set up in parallel and a test is considered abnormal if the patient’s cells demonstrate consistently 50% or less of the cell death observed in the control. Additionally, shipped samples should be accompanied by a shipping control.

The demonstration of germline or somatic deleterious mutations in FAS, FASLG, or CASP10 is now considered a diagnostic criterion. Patients with germline CASP8 and somatic NRAS mutations are now classified separately (Table 2). Gene sequencing is generally available by selected commercial laboratories; however, as polymorphisms in FAS are not uncommon, a diagnostic mutation should be based on prior identification of the mutation linked to a diagnosis of ALPS or a proven functional consequence of the change in association with a new mutation. Existing databases of pathogenic FAS mutations are publicly available and can be used for diagnostic help (NCBI NIH ALPS website http://www3.niaid.nih.gov/topics/ALPS/). However, isolated discovery of a heterozygous Fas mutation in a healthy relative of a patient with ALPS is of clinically uncertain significance at this time.
Based on recent data, the presence of elevated TCRαβ⁺-DNT cells coupled to high serum or plasma levels of either interleukin (IL)-10, IL-18, soluble FAS ligand (sFASL) or vitamin B12 can accurately predict the presence of germline or somatic FAS mutations\textsuperscript{24-27}. These biomarkers can predict a FAS mutation with a post-test probability ranging from 85 to 97%, depending on the biomarker used and the number of TCRαβ⁺-DNT cells\textsuperscript{24-27}. Given this high specificity, these biomarkers were also incorporated in the diagnostic criteria and their use should greatly facilitate the diagnosis in settings without access to advanced genetic analysis or functional testing.

Two common presenting features of ALPS, autoimmune cytopenias and hypergammaglobulinemia, are now incorporated as diagnostic criteria. A recent publication suggests that their presence in patients with lymphoproliferation and elevated TCRαβ⁺-DNT cells indicates a very high likelihood of ALPS and these patients should be referred for further testing\textsuperscript{24}. Although autoimmune manifestations of ALPS are typically limited to hematopoietic elements that lead to multilineage cytopenias, occasionally other organs, including liver and kidneys, may also be affected\textsuperscript{29}.

Lymph node pathological findings initially described by Jaffe and colleagues are characteristic of ALPS and are included as a secondary accessory diagnostic criterion\textsuperscript{30}. These findings include paracortical expansion due to infiltration by polyclonal TCRαβ⁺-DNT cells accompanied by follicular hyperplasia and polyclonal plasmacytosis\textsuperscript{30}. Marked TCRαβ⁺-DNT cell infiltration in some cases can lead to architectural effacement of lymph nodes with infiltration of bone marrow and spleen, leading in some instances to an erroneous diagnosis of peripheral T-cell lymphoma. The diagnostic workup should include flow cytometric or immunohistochemical evaluation of T-cells for CD3, CD4, CD8, CD57, CD45RO, and CD45RA using standardized laboratory methods\textsuperscript{31}. Utilizing flow cytometry αβ and γδ T-cells should be distinguished, with gating for CD4 and CD8 performed on the respective populations. In addition, polymerase chain reaction studies of T-cell receptor gene rearrangement should indicate the absence of a clonal T cell population in ALPS.

The final secondary accessory criterion is a positive family history for non-malignant and non-infectious lymphadenopathy/splenomegaly with or without
autoimmunity, since many ALPS patients have family members with similar clinical histories.

**MODIFICATIONS TO THE CLASSIFICATION OF ALPS AND RELATED DISORDERS**

**Rationale**

The molecular classification of ALPS has seen many recent additions over time \(^{14-19}\), leading to somewhat chaotic nomenclature (Table 3). An ideal classification system should not only standardize the nomenclature among different centers but also easily accommodate future discoveries. This revision introduces extensive modifications to the previously used classification of ALPS and related disorders, as summarized below (Table 3).

**Recommendations**

For simplicity, numbers should no longer be used when classifying ALPS based on the genetic defect (Table 3). Patients harboring germline homozygous, or heterozygous mutations in *FAS*, previously classified as ALPS Type 0 and Ia, respectively, are now unified under ALPS-FAS. Similarly, patients with somatic FAS mutations should be classified as ALPS-sFAS; patients harboring Fas ligand mutations should be classified as ALPS-FASLG; and patients with caspase-10 mutations classified as ALPS-CASP10.

Patients who fulfill diagnostic criteria for ALPS but in whom no genetic diagnosis can be determined should now be classified as ALPS-U (undetermined), instead of ALPS Type III. We expect new genetic defects to be discovered in this group of patients with further research. Despite the lack of a genetic diagnosis, our current understanding is that this group of patients has a clinical course that is similar to other ALPS patients, except that there is no evidence yet of an increased incidence of lymphoma (VK Rao, unpublished findings). The diagnostic flow chart shown in Figure 1 should help the readers to navigate among different ALPS subtypes in their clinical practice.
## Table 3. Revised Classification of ALPS

<table>
<thead>
<tr>
<th>Previous Nomenclature</th>
<th>Revised Nomenclature</th>
<th>Gene</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALPS Type 0</td>
<td>ALPS-FAS</td>
<td>FAS</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline homozygous mutations in FAS.</td>
</tr>
<tr>
<td>ALPS Type Ia</td>
<td>ALPS-FAS</td>
<td>FAS</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline heterozygous mutations in FAS.</td>
</tr>
<tr>
<td>ALPS Type Im</td>
<td>ALPS-sFAS</td>
<td>FAS</td>
<td>Patients fulfill ALPS diagnostic criteria and have somatic mutations in FAS.</td>
</tr>
<tr>
<td>ALPS Type Ib</td>
<td>ALPS-FASLG</td>
<td>FASLG</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline mutations in FAS ligand.</td>
</tr>
<tr>
<td>ALPS Type IIa</td>
<td>ALPS-CASP10</td>
<td>CASP10</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline mutations in caspase 10.</td>
</tr>
<tr>
<td>ALPS Type III</td>
<td>ALPS-U</td>
<td>Unknown</td>
<td>Patients meet ALPS diagnostic criteria; however, genetic defect is undetermined (no FAS, FASL or CASP10 defect).</td>
</tr>
</tbody>
</table>
Figure 1. Suggested algorithm for diagnostic work up for patients with suspected ALPS.
Classification of ALPS-related disorders

Patients with mutations in the gene encoding for caspase-8 (CASP8) present with a syndrome of lymphadenopathy and splenomegaly, marginal elevation of DNTs, and defective FAS-induced lymphocyte apoptosis, were previously classified as ALPS Type IIb\textsuperscript{15}. However, in contrast to other ALPS cases, these patients also demonstrate defective T, B, and NK cell activation, with consequent recurrent bacterial and viral infections\textsuperscript{15,32}. Given the distinct phenotype, the previously defined term Caspase-Eight Deficiency State (CEDS) is now included to describe this disorder (Table 4)\textsuperscript{29}.

Table 4. Revised Classification of ALPS-related disorders

<table>
<thead>
<tr>
<th>Previous Nomenclature</th>
<th>Revised Nomenclature</th>
<th>Gene</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALPS Type IIb</td>
<td>CEDS</td>
<td>CASP8</td>
<td>Patients present with lymphadenopathy and/or splenomegaly, marginal DNT elevation, recurrent infections and germline mutations in caspase 8.</td>
</tr>
<tr>
<td>ALPS Type IV</td>
<td>RALD</td>
<td>NRAS</td>
<td>Patients present with autoimmunity, lymphadenopathy and/or splenomegaly, elevated or normal DNTs and somatic mutations in NRAS.</td>
</tr>
<tr>
<td>DALD</td>
<td>DALD</td>
<td>Unknown</td>
<td>Patients present with autoimmunity, lymphadenopathy and/or splenomegaly, normal DNTs and defective in vitro FAS-mediated apoptosis.</td>
</tr>
<tr>
<td>XLP1</td>
<td>XLP1</td>
<td>SH2D1A</td>
<td>Patients present with fulminant Epstein-Barr virus infection, hypogammaglobulinemia or lymphoma.</td>
</tr>
</tbody>
</table>

CEDS, caspase-eight deficiency state; RALD, RAS-associated autoimmune leukoproliferative disease; DALD, Dianzani autoimmune lymphoproliferative disease; X-linked lymphoproliferative syndrome. 

The clinical syndrome of autoimmune phenomena, lymphocyte accumulation, and somatic mutations in NRAS, previously designated ALPS Type IV, is now reclassified
under a new nosologic entity termed RALD, for RAS-associated autoimmune leukoproliferative disease (Table 4)\textsuperscript{19}. The main rationale for this change was the recognition of two additional patients with somatic \textit{NRAS} mutations who did not demonstrate elevated TCR\(\alpha\beta\) DNT cells (Oliveira JB and Bleesing JJ, unpublished observations). Additionally, these patients presented with atypical features such as elevations in cells of myeloid origin (monocytosis and granulocytosis), and demonstrated partial overlap with juvenile myelomonocytic leukemia (JMML) as well as lymph node histopathology not typical of ALPS (Oliveira JB, unpublished observations).

No nomenclature modifications are suggested for the ALPS-related clinical syndrome known as DALD (Dianzani Autoimmune Lymphoproliferative Disease)\textsuperscript{33}, characterized by autoimmunity, lymphadenopathy and/or splenomegaly, and defective in vitro Fas-mediated lymphocyte apoptosis, without elevation in TCR\(\alpha\beta\) DNT cells. The genetic defect is not known, but an inherited component is suggested based on the defective FAS function displayed by relatives of these patients. Patients may display a wide range of autoimmune manifestations and an increased risk of cancer has been reported\textsuperscript{34,35}. Finally, the X-linked lymphoproliferative disease (XLP1), a genetic immunodeficiency caused by mutations or deletions in the \textit{SH2D1A} gene, can be included in the spectrum of ALPS-like disorders. These patients frequently display defective apoptosis in response to T cell receptor (TCR) restimulation and this pathway appears to be essential for constraining effector T cell expansion and preventing immunotoxicity\textsuperscript{36,37}.

**CONCLUSION**

The modifications in the diagnostic criteria and classification system introduced here should facilitate diagnosis in locations without access to advanced testing, streamline diagnostic work up of patients, standardize nomenclature among different centers and allow easy inclusion of newly discovered genetic defects resulting in classical ALPS.

**Acknowledgments**

We thank all the participants of the NIH ALPS workshop, conducted on September 21-22, 2009, which led to further discussions and deliberations leading to the preparation of
this document. This research was supported by the Intramural Research Program of the NIH (NIAID, NCI, CC) and funding for the ALPS Workshop was provided by NIH Office of the Rare Diseases (ORD) and Division of Intramural Research, NIAID. Authors also wish to thank Ugo Ramenghi, Andrew Snow, and Michael Sneller for reviewing this manuscript.

References