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New Advances in the Diagnosis and Treatment of Autoimmune Lymphoproliferative Syndrome (ALPS)

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Abstract

Purpose of Review—Autoimmune Lymphoproliferative Syndrome (ALPS) is a disorder of disrupted lymphocyte homeostasis, resulting from mutations in the Fas apoptotic pathway. Clinical manifestations include lymphadenopathy, splenomegaly, and autoimmune cytopenias. A number of new insights have improved the understanding of the genetics and biology of ALPS. These will be discussed in this review.

Recent Findings—A number of key observations have been made recently that better define the pathophysiology of ALPS, including the characterization of somatic *FAS* variant ALPS, the identification of haploinsufficiency as a mechanism of decreased Fas expression, and the description of multiple genetic hits in *FAS* in some families that may explain the variable penetrance of the disease. In addition, ALPS has been shown to be a more common condition, as patients diagnosed with other disorders, including Evans syndrome and common variable immune deficiency have been found to have ALPS. Finally, the treatment of the disease has changed as splenectomy and rituximab have been shown to have unexpected ALPS specific toxicities, and mycophenolate mofetil and sirolimus have been demonstrated to have marked activity against the disease.

Summary—Based on novel advances the diagnostic algorithm and recommended treatment for ALPS have changed significantly, improving quality of life for many patients.

Keywords

Autoimmune Lymphoproliferative Syndrome; cytopenias; secondary malignancy; FAS; RAS; Evans syndrome; mTOR

Introduction

Autoimmune Lymphoproliferative Syndrome (ALPS) is a disorder of abnormal lymphocyte survival caused by dysregulation of the FAS apoptotic pathway. Patients with ALPS develop chronic non-malignant lymphoproliferation, autoimmune disease, and secondary malignancies. ALPS was first described in the 1990s, and since its discovery a number of significant advances have been made that have changed diagnosis and treatment of ALPS and have better defined its pathophysiology. ALPS was originally thought to be extremely rare but may be more common as more cases are being diagnosed with increasing clinician awareness. This article reviews the diagnosis and treatment of ALPS, focusing on recent scientific advances.

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Pathophysiology and Genetics

ALPS is a syndrome defined by a defect in the Fas apoptotic pathway (Figure 1).[1] In order to downregulate the normal immune response, activated B and T lymphocytes increase Fas expression, and activated T lymphocytes increase expression of Fas-ligand.[2] Fas and Fas-ligand interact through the Fas-activating death domain (FADD), triggering the caspase cascade, culminating in DNA degradation, proteolysis and apoptosis.[3] Defective apoptosis can lead to lymphoproliferation, autoimmunity, and cancer.[4,5] Over 70% of patients with ALPS have identifiable mutations in Fas pathway genes. Most patients have germline (60–70% of patients) or somatic mutations (10% of patients) in *FAS (TNFRSF6).* Rarely, patients have mutations in *FASL (TNFSF6,* <1% of patients) and *CASP10* (2–3% of patients).[6] Twenty to 30% of patients have no identifiable mutation.[6] Recently, an international consensus conference at the NIH redefined ALPS nomenclature, using a gene-based classification (Table 1).[7]

Germline mutations in *FAS* are usually inherited in an autosomal dominant fashion.[8] Most often *FAS* defects are dominant negative heterozygous missense mutations in the intracellular death domain (exons 7–9), with the mutated allele inhibiting the function of the wild-type allele. Approximately 30% of ALPS causative mutations affect the extracellular domain. Recently, Keuhn and colleagues established these are mostly nonsense or frameshift mutations resulting in haploinsufficiency.[9] Dominant negative mutations typically lead to absence of FAS activity, whereas haploinsufficient mutations usually lead to decreased activity. As such, disease penetrance appears to be much higher in families with dominant negative intracellular mutations compared with haploinsufficient extracellular mutations.[9–11] ALPS mutations have highly variable expressivity, and mutation type is not predictive of disease manifestations or laboratory biomarkers. The only reported association is a higher incidence of secondary lymphomas in patients with dominant negative intracellular mutations.[9] Of note, the total number of reported patients with ALPS-associated lymphoma is small, and this observed difference may result from selection bias.

A significant subset of ALPS patients have somatic *FAS* mutations, primarily limited to the non-thymic double negative T cell (DNT) compartment. DNTs (phenotype, CD3+, CD4−, CD8−, TCRα/β+) are a subset of T cells normally found at small percentages in the peripheral blood that are markedly elevated in patients with ALPS. The elevated DNTs in ALPS were originally considered an epiphenomenon; however, they may drive abnormal B cell activity and subsequent autoimmunity.[12] The recent description of somatic ALPS is a strong argument that DNTs play a role in ALPS pathogenesis. ALPS patients with germline and somatic-variant *FAS* mutations are phenotypically similar in both disease manifestations and laboratory abnormalities.[13,14] Predictably, the primary difference is that patients with germline *FAS* mutations typically present at a younger age than their somatic counterparts. Causative somatic mutations in *CASP10* and *FASL* have not been described.

FAS mutations have variable penetrance, as ALPS patients often have family members with the same genetic alterations and an absent or very mild clinical phenotype [15,16]. This suggests a second “hit” is required for disease, such as additional genetic mutations and/or environmental triggers. Recently, Magerus-Chatinet and colleagues observed that disease penetrance can be explained in some families as a consequence of multiple *FAS* mutations. [17] They identified seven ALPS patients who inherited heterozygous *FAS* mutations and also acquired a somatic alteration in the second *FAS* allele. Family members with only germline *FAS* mutations were asymptomatic. Multiple abnormalities in *FAS* may explain the variable penetrance in some families; however, patients may acquire mutations in other cooperating genes as the second event.

Clinical Manifestations and Diagnosis

Lymphoproliferation is the most common clinical manifestation in ALPS, presenting as lymphadenopathy, splenomegaly, and/or hepatomegaly.[6,18–20] Most patients develop lymphoproliferation at a young age (median 11.5 months) that varies from mild to severe enough to compromise vital organs. [21] Autoimmunity is the second most common finding and is the most likely to require medical intervention. Over 70% of patients develop autoimmune disease, most commonly immune-mediated cytopenias, which can affect erythrocytes (autoimmune hemolytic anemia), platelets (immune thrombocytopenia) or neutrophils (autoimmune neutropenia).[15] Severity ranges from asymptomatic laboratory abnormalities to severe, chronic, and debilitating destruction of multiple cell lineages.[6] Other autoimmune manifestations are less frequent, although ALPS autoimmunity can affect virtually any organ, and patients can develop nephritis, gastritis, hepatitis, urticaria, arthritis, colitis, and pulmonary fibrosis, similar to systemic lupus erythematosis.[10,22,23] While autoimmune neurologic disease is a rare manifestation of ALPS, we have diagnosed severe autoimmune neurologic complications in three patients, including autoantibody-positive autoimmune cerebellar ataxia, transverse myelitis, and Guillain-Barre syndrome (unpublished observation). All three patients responded to systemic immune suppression.

ALPS patients have an increased risk of secondary malignancies, most commonly EBER+ non-Hodgkin lymphoma.[10,15] The risk is estimated to be 10–20% and is most prevalent in *FAS*-mutant ALPS.[16] Unaffected family members with *FAS* mutations are also predisposed to malignancy, consistent with observations that somatic mutations in *FAS* family genes are highly prevalent in lymphomas in the general population.[24] Identifying malignancy can be difficult as clinical manifestations of ALPS mirror lymphoma. No imaging modality, including FDG-PET, can accurately distinguish benign from malignant lymphoproliferation because rapidly proliferating cells in ALPS have high FDG uptake.[25] Unlike carcinomas and other solid tumors, early diagnosis of lymphoma does not change clinical outcome. Accordingly, based on the very high false-positive rate, our group does not perform routine serial imaging of ALPS patients. Rather, we investigate for malignancy if patients develop constitutional symptoms or have a significant change in disease pattern. ALPS lymphadenopathy consists primarily of polyclonal expansion of abnormal cells. Progression to lymphoma is associated with monoclonal expansion of malignant lymphocytes. Testing for clonality in lymphocyte subsets can sometimes help distinguish benign from malignant disease.

Until 2010, diagnosis of ALPS required meeting three mandatory criteria: (1) chronic (> 6 months) non-malignant lymphoproliferation; (2) elevated peripheral blood DNTs; and (3) defective in vitro Fas-mediated apoptosis (Table 2). The first two criteria remain mandatory for diagnosis. DNTs are assessed by flow cytometry of peripheral blood or lymphoid tissue. DNTs should only be tested in an experienced clinical laboratory and must include testing for the TCRα/β receptor, as other non-pathogenic lymphocyte subsets are CD3+/CD4−/CD8−. DNT test results must be interpreted carefully, as the normal range can vary between laboratories. Finally, lymphopenic patients and those on high dose steroids or sirolimus should not undergo DNT testing due to risks of false positives or negatives, respectively.

In order to test for defective in vitro Fas-mediated apoptosis, a patient's peripheral blood mononuclear cells are isolated, activated with mitogen, and expanded with interleukin-2 (IL-2) in culture for 10–28 days.[4] Normally, mitogen activation and T cell expansion upregulate the Fas pathway. Subsequent exposure of normal T cells to anti-Fas immunoglobulin M (IgM) monoclonal antibody in vitro leads to rapid apoptosis.[26] ALPS patients' T cells do not die after exposure.[27] This is an elegant but expensive and labor-intensive assay, requiring samples from patients and unaffected controls. DNTs do not

survive in routine culture; this assay only identifies Fas defects in non-DNT T cells. Patients with somatic-variant ALPS or *FASL* mutations usually have normal apoptosis assays.

A number of recent studies have identified reliable biomarkers for ALPS, including elevated serum vitamin B12, soluble Fas ligand, IL-10, and IL-18.[13,28] These biomarkers are very specific for ALPS in patients with lymphoproliferation and elevated DNTs and are most predictive for *FAS*-variant (germline or somatic) ALPS. Our group established that a combination of autoimmune cytopenias and hypergammaglobulinemia are very predictive of ALPS in patients with lymphoproliferation and elevated DNTs.[29]

The recent international consensus conference led to a new diagnostic algorithm that includes the three previously mandatory criteria but also allows for the diagnosis of ALPS with genetic testing, biomarkers, family history, and/or histopathology (Table 2).[7] The new algorithm makes a nomenclature distinction between definitive versus probable diagnoses; however, the published consensus statement recommends that patients with definitive and probable ALPS be treated the same clinically. A number of clinical laboratories can perform specialized testing for DNTs, FAS and other gene mutations, and biomarkers, including IL-10 and sFASL. The Diagnostic Immunology and Molecular Genetics laboratories at Cincinnati Children's Hospital (Cincinnati, OH) provide the most comprehensive assortment of testing. Other laboratories that offer a number of specialized tests, include the Children's Hospital of Philadelphia (Philadelphia, PA), and GeneDX (Gaithersburg, MD).

Differential Diagnosis

ALPS patients have highly heterogeneous phenotypes with clinical findings that overlap with malignant, infectious, autoimmune, and rheumatologic conditions. Several lymphoproliferative disorders, including Castleman disease, Rosai-Dorfman disease, X-linked lymphoproliferative disease (XLP), Dianzani Autoimmune Lymphoproliferative Disease (DALD), Kikuchi-Fujimoto disease, Caspase 8 deficiency syndrome (CEDS), and Ras-associated leukoproliferative disorder (RALD) can present with clinical features similar to ALPS. As these disorders are often distinguishable by histopathology, most patients should undergo tissue biopsy (bone marrow and/or lymph node) at initial presentation.

Patients with mutations in *CASP8* were originally classified as having ALPS because caspase 8 and caspase 10 have similar functions, and *CASP8* mutant patients present with lymphadenopathy and defective Fas-mediated apoptosis [30]. While patients with ALPS primarily have apoptotic defects in T lymphocytes, patients with *CASP8* mutations have profound apoptotic defects in B, T, and NK lymphocytes [30]. Patients with *CASP8* mutations are predisposed to significant mucocutaneous infections with herpes virus [30]. Thus, patients with *CASP8* mutations are now considered to have a distinct disease termed CEDS.

Rosai-Dorfman (RD) disease is a histiocytic disorder with considerable phenotypic overlap with ALPS. RD is diagnosed by histopathology. Emperipolesis (lymphophagocytosis) was thought to be pathognomonic for RD; however, lymph node biopsies from ALPS patients can demonstrate emperipolesis, as well as other RD features.[31] Accordingly, testing for ALPS should be considered in any child diagnosed with RD.

X-linked lymphoproliferative disorder (XLP) is a rare disorder characterized by a dysregulated T and NK cell-mediated immune response to EBV infection, most commonly as a consequence loss of SLAM-associated protein (SAP) because of mutations in *SH2D1A*. Patients with XLP can present with fulminant mononucleosis, hematophagocytic syndrome, aplastic anemia and/or aggressive lymphoproliferative disease. Recently, SAP-deficient T

cells collected from XLP patients were demonstrated to have defective restimulation-induced apoptosis.[32] Thus, XLP can be classified as an ALPS-like disorder of abnormal lymphocyte apoptosis.

Ras-associated leukoproliferative disorder (RALD) is a newly described lymphoproliferative disorder characterized by impaired cytokine withdrawal-induced apoptosis in T cells due to gain of function somatic mutations in *RAS* family genes (*KRAS* and *NRAS*).[33–35] Fewer than 10 patients with RALD have been reported, resulting in limited knowledge of the clinical manifestations of the disease. Common features include lymphoproliferation, autoimmune cytopenias, and hypergammaglobulinemia. DNTs may be mildly elevated in RALD but are usually normal. As in juvenile myelomonocytic leukemia (JMML; a myeloproliferative disorder characterized by *RAS* mutations), myeloid cells from these patients may demonstrate hypersensitivity to GM-CSF. Recent literature suggests RALD is a non-malignant disease based on its indolent nature; however, no published studies have described whether the abnormal cells in RALD are clonal. Secondary cancers and unexplained sudden death have been described in RALD and somatic gain-of-function mutations in *RAS* family genes are extremely common in cancer. Thus, more studies are needed to determine if RALD is a benign lymphoproliferative disorder, a pre-malignant condition, or a malignant state.

Patients with common variable immunodeficiency (CVID) can present with lymphadenopathy and autoimmune disease mirroring ALPS.[36] In addition, a small subset of ALPS patients have co-morbid CVID. Specific testing for ALPS, including DNT analysis, apoptosis assays, and genetic testing, may aid in distinguishing the conditions. Arguably, any patient with CVID and secondary autoimmune cytopenias should be tested for ALPS.

Evans syndrome (ES), defined by autoimmune destruction of at least two hematologic cell types, can also have a similar presentation to ALPS [37]. Recently, our group demonstrated in an multi-institutional trial that a significant percentage of children diagnosed with ES have ALPS.[26,29] There was likely selection bias, as not all ES patients were captured at each participating institution, and a similar prevalence of ALPS may not be found among ES patients in other populations. A recent comprehensive analysis of children with autoimmune hemolytic anemia in France, including a large cohort of ES patients, did not find a high incidence of ALPS.[38] ALPS patients were identified in the study but at a lower rate than in US studies. Of note, not all patients were tested for ALPS, and only ES patients with autoimmune hemolytic anemia were included.

Treatment

While some ALPS patients require no treatment, many require immunosuppression, particularly to treat cytopenias. Some patients develop organ compromise from lymphoproliferation, requiring medical intervention. Most patients respond to short corticosteroid pulses. [39] While appropriate for periodic disease flares, corticosteroids are too toxic for use in chronic disease. As recently as five years ago, medication choices for ALPS autoimmunity did not differ from other autoimmune conditions. Recent studies have established that some therapies commonly used in other conditions are relatively contraindicated, while other less common therapies are very effective in ALPS.

Rituximab and splenectomy are often the treatments of choice in refractory autoimmune cytopenias in children. Rituximab can lead to prolonged, clinically significant hypogammaglobulinemia when used in ALPS. Thus, alternative immune suppressants should be tried before using rituximab, if possible.[40,41] Rituximab is active in many ALPS patients, and can be used when other agents are ineffective or not tolerated. Patients

should be advised that they may require prolonged IVIgG replacement therapy. Patients with ALPS have a very high risk of developing post-splenectomy sepsis, even with antibiotic prophylaxis and vaccination.[42,43] Accordingly, splenectomy should be avoided except in the case of uncontrolled hypersplenism that fails other medical management.

The two best-studied and most effective non-steroid agents used in children with ALPS are mycophenolate mofetil (Cellcept, MMF) and sirolimus (rapamycin). MMF inactivates inosine monophosphate, a key enzyme in purine synthesis required for lymphocyte proliferation [44,45]. Over thirty patients treated with MMF have been reported, and over 80% of these patients demonstrated measurable improvements in autoimmune disease [44,46,47]. MMF does not improve lymphoproliferation or reduce DNTs. Many of these patients had only partial responses, and some relapsed. MMF is a well-tolerated medication with side effects including neutropenia and diarrhea. In Europe, another purine synthesis inhibitor, mercaptopurine, is commonly used instead of MMF.

Sirolimus (rapamycin), a mammalian target of rapamycin (mTOR) inhibitor, has also been studied extensively in ALPS. We hypothesized that targeting the PI3K/mTOR/Akt signaling pathway may be effective in ALPS (Figure 2). In support, the mTOR signaling pathway is upregulated in murine ALPS (our unpublished results), and sirolimus is very active in this disease model, demonstrating superior efficacy than other therapies including MMF.[48] We subsequently opened a clinical trial that has examined 13 patients thus far, and continues to enroll (NCT00392951).[49,50] Our early results indicate that sirolimus is uniquely active in ALPS with most patients showing rapid, complete responses (unpublished data).[49] The majority of patients had failed other treatments, often involving multiple agents, including corticosteroids and MMF. In these patients, sirolimus inhibited both autoimmune disease and lymphoproliferation, and in most patients, eliminated the abnormal DNTs. These results have been confirmed in independent studies.[51,52] Based on our observation that the mTOR signaling pathway is abnormally activated in ALPS, treating ALPS patients with sirolimus is a form of targeted therapy that is well tolerated with little toxicity. Side effects include hypercholesterolemia, hypertension, and mucositis.[53] Of note, we continue to offer MMF as a first line for chronic treatment, as sirolimus requires therapeutic drug monitoring. For patients with more aggressive disease or in those with symptomatic lymphoproliferation, we use sirolimus as first line.

Conclusion

In summary, over the past decade a number of remarkable insights have been made that improve understanding of the underlying pathogenesis of ALPS. Through international collaborations, the diagnosis and treatment of ALPS has changed significantly in a short time, making it easier for physicians to diagnosis the disease and improving quality of life for patients.

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Key Points

1. The diagnostic algorithm for ALPS has recently changed with less focus on a functional research based apoptosis assay and more reliance on genetic testing and biomarkers.
2. A combination of multiple mutations (acquired and inherited) in ALPS causative genes may help explain low disease penetrance in families
3. Autoimmune disease and lymphoproliferation are treated differently in ALPS than similar disorders. Rituximab and splenectomy should be avoided. Mycophenolate mofetil and sirolimus should be considered in patients with chronic disease.

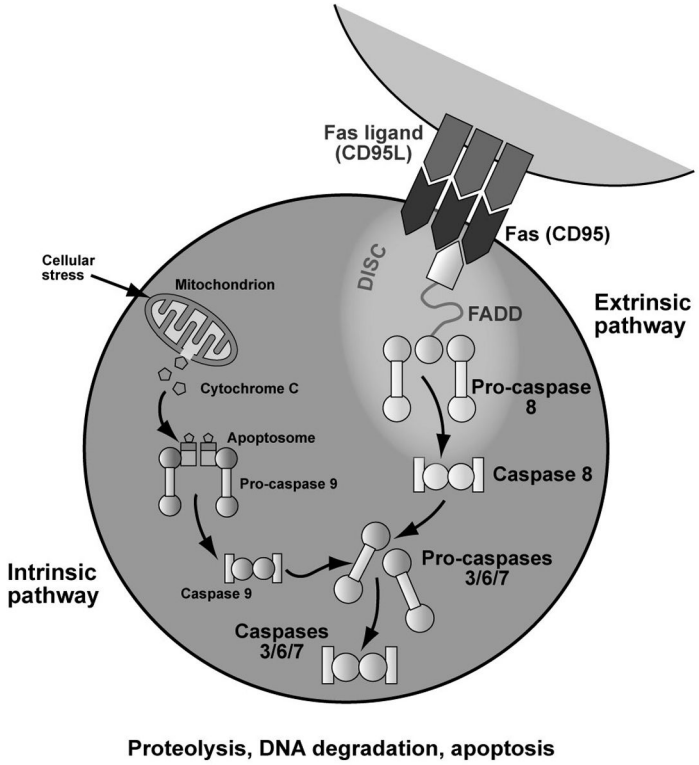

Figure 1. Fas apoptotic pathway

ALPS is caused by defective Fas-mediated apoptosis. Normally, as part of the down-regulation of the immune response, activated B and T lymphocytes increase Fas expression, and activated T lymphocytes increase expression of Fas ligand. Fas and Fas ligand interact which activates the Fas-associated death domain (FADD) and triggers the caspase cascade, culminating in cellular apoptosis. Fas-mediated signaling is part of the extrinsic apoptotic pathway, because it is activated through the interactiong of cell surface death receptors. In contrast, the intrinsic apoptotic pathway is activated by cellular stressors that lead to decreased mitochondrial membrane permeability with release of apoptosis-inducing substances. © Sue Seif, MA (used with permission).

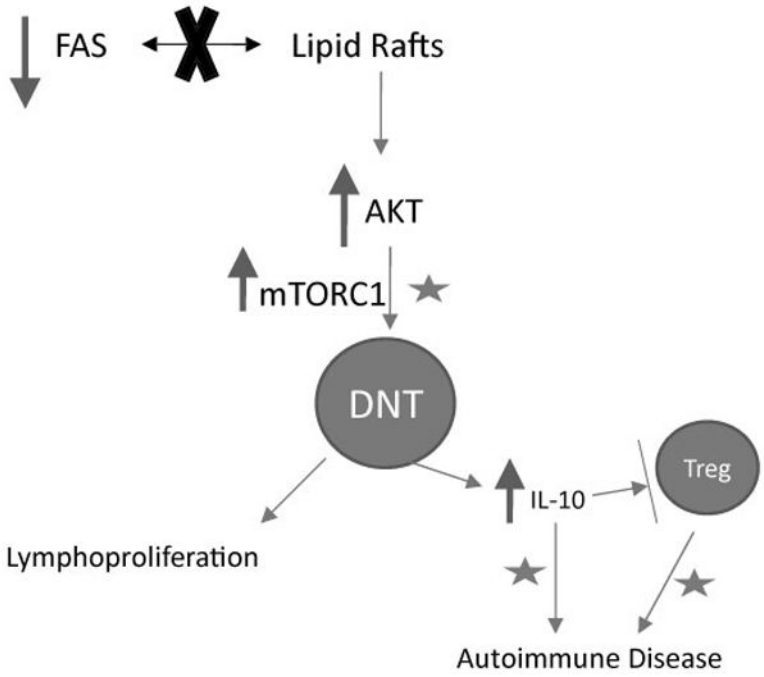

Figure 2. Hypothetical Model of Dysregulation of PI3K/Akt/mTOR Signaling in ALPS

ALPS is a defined by defects in the Fas apoptotic pathway; however, individuals with causative mutations do not always manifest disease symptoms. Fas must be recruited into lipid rafts in order to stimulate apoptosis. We hypothesize that in ALPS the defects in the Fas pathway lead to decreased distribution of Fas receptor into lipid rafts, thereby activating the serine threonine kinase Akt. In support, Akt activity and distribution of Fas into lipid rafts are inversely correlated in some lymphocyte subsets.[54] Constitutive activation of Akt stimulates the mTOR pathway, causing uncontrolled proliferation of the double negative T cells (DNTs) with consequent lymphadenopathy and splenomegaly. These DNTs also secrete IL-10, causing autoimmunity.High levels of IL-10 down-regulate TGF-β producing T_{regs}, a subset of T lymphocytes that can regulate immune tolerance.[55,56] We observe abnormally low T_{reg} numbers in ALPS (unpublished). Moreover, low levels of TGF-β producing T-cells can cause an “ALPS-like” disease in mice.[57] Thus, the T_{reg} dysregulation may exacerbate the systemic autoimmunity. Accordingly, mTOR inhibitors, including sirolimus may alleviate ALPS through combined mechanisms indicated by stars. First, mTOR inhibitors can directly target the dysregulated PI3K/Akt/mTOR signaling axis and reduce accumulation of abnormal DNTs. Second, mTOR inhibitors may block signaling through IL-10.[58,59] Finally, in contrast to most immunosuppressants, mTOR inhibitors can spare T_{regs}, and thereby help to reset the immune imbalance.

Table 1

Revised classification of ALPS and ALPS-related diseases. (Adapted with permission from [7])

ALPS types		
New	**Old**	**Gene**
ALPS-FAS	ALPS Ia	*FAS* (germline)
ALPS-sFAS	ALPS Im (or ALPS Is)	*FAS* (somatic)
ALPS-FASL	ALPS Ib	*FASL*
ALPS-CASP10	ALPS IIa	*CASP10*
ALPS-U	ALPS III	Unknown/Undefined
ALPS-related diseases (formally classified as ALPS)		
New	**Old**	**Gene**
CEDS	ALPS IIb	*CASP8*
RALD	ALPS IV	*NRAS, KRAS*

CEDS: Caspase 8 deficiency state; RALD: Ras-associated leukoproliferative disease

Table 2

Diagnostic Criteria for ALPS. (Adapted with permission from [7])

Old criteria
Required
1. Chronic non-malignant lymphoproliferation
2. Elevated peripheral blood DNTs
3. Defective in vitro Fas mediated apoptosis
New criteria
Required
1. Chronic non-malignant lymphoproliferation (<6 months lymphadenopathy and/or splenomegaly)
2. Elevated peripheral blood DNTs
Accessory
Primary
1. Defective in vitro Fas mediated apotosis (in 2 separate assays)
2. Somatic or germline mutation in ALPS causative gene (FAS, FASL, CASP10)
Secondary
1. Elevated biomarkers (Any of following) **a.** Plasma sFASL >200pg/ml **b.** Plasma IL-10 >20pg/ml **c.** Plasma or serum vitamin B12 >1500ng/L **d.** Plasma IL-18 >500pg/ml
2. Immunohistochemical findings consistent with ALPS as determined by experienced histopathologist
3. Autoimmune cytopenias AND polyclonal hypergammaglobulinemia
4. Family history of ALPS or nonmalignant lymphoproliferation
Definitive Diagnosis: Required plus one primary accessory criteria Probable Diagnosis: Required plus one secondary accessory criteria Of note, probable and definitive ALPS should be treated the same in the clinic