Autoimmune lymphoproliferative syndrome

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Autoimmune lymphoproliferative syndrome arises early in childhood in people who inherit mutations in genes that mediate lymphocyte apoptosis, or programed cell death. In the immune system, antigen-induced lymphocyte apoptosis maintains immune homeostasis by limiting lymphocyte accumulation and minimizing reactions against self-antigens. In autoimmune lymphoproliferative syndrome, defective lymphocyte apoptosis manifests as chronic, nonmalignant adenopathy and splenomegaly; the expansion of an unusual population of $CD4^{-}CD8^{-}$ T cells; and the development of autoimmune disease. Most cases of autoimmune lymphoproliferative syndrome involve heterozygous mutations in the lymphocyte surface protein Fas (CD95, Apo1) that impair a major apoptotic pathway. Prospective evaluations of patients and their families have revealed an ever-expanding spectrum of autoimmune lymphoproliferative syndrome and its major complications. Curr Opin Rheumatol 2003, 15:417–421

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Abbreviations

ALPS	autoimmune lymphoproliferative syndrome
ITP	idiopathic thrombocytopenic purpura
TNFRSF6	Tumor Necrosis Factor Receptor SuperFamily-6

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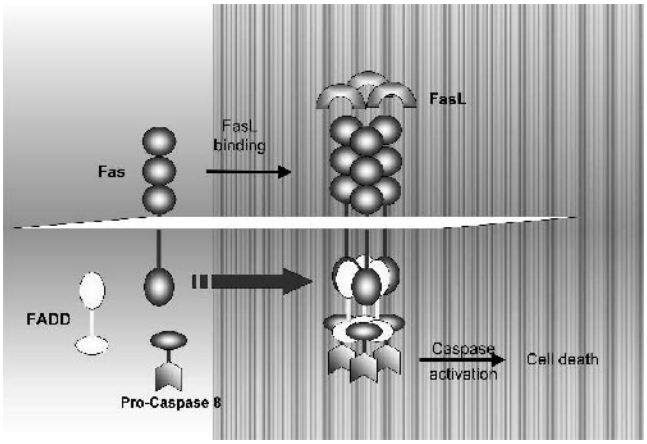
Mature lymphocytes undergo a life cycle of activation and immune effecter responses followed by apoptosis, or programed cell death. In the setting of lymphocyte activation, apoptosis permits the constant reshaping and fine trimming of the immune repertoire. Thus, apoptosis maintains immune homeostasis by limiting lymphocyte accumulation and minimizing reactions against self-antigens. Antigen-provoked apoptosis of lymphocytes is mediated by members of the tumor necrosis factor family of cytokines and their receptors. The chief member of the family involved in lymphocyte apoptosis is the Fas receptor protein (also known as CD95 orApo1) and its ligand (CD95L). The interaction between Fas and Fas ligand triggers a dedicated molecular pathway leading to cell death [1]. This pathway begins with the aggregation of Fas proteins into a homotrimer that then recruits an adapter protein referred to as *Fas-associated death domain* and the aspartate-specific cysteine protease caspase-8 (or, in some instances, caspase-10). This molecular complex in turn initiates cleavage and activation of downstream proteins in a cascade that ultimately results in cell death (Fig. 1).

The *in vivo* importance of this pathway in maintaining lymphocyte homeostasis is illustrated by the disease long recognized to develop in *lpr* and *gld* strains of mice that are genetically deficient in expression of Fas or Fas ligand, respectively. Mice homozygous for either of these mutations develop hypergammaglobulinemia, autoantibody production, glomerulonephritis, massive lymphoid hyperplasia, and expansion of an unusual population of T cells that express the α/β T-cell receptor but do not express either CD4 or CD8 (double-negative T cells) [2].

The relevance of the Fas apoptotic pathway to human disease was postulated in 1992 with the authors' description of two children with massive, nonmalignant lymphoid hyperplasia and autoimmune disease [3]. We referred to this novel syndrome as *autoimmune lymphoproliferative syndrome* (ALPS). Subsequently it was demonstrated that patients with ALPS exhibit defective lymphocyte apoptosis that in most cases is caused by inherited heterozygous mutations in the Fas gene [4–6]. Mutations in Fas ligand, caspase-8, or caspase-10 have been identified in a minority of residual cases lacking Fas mutations. Thus, ALPS represents a heritable failure of apoptotic mechanisms with a consequent accumulation of lymphoid mass and autoreactive lymphocytes. It is defined operationally by clinical and laboratory findings summarized in Table 1. Autoimmunity is evident at

Figure 1. Schematic representation of the Fas apoptosis pathway



FADD, Fas-associated death domain; FasL, Fas ligand.

some point in the majority of patients and can be of considerable severity and chronicity.

Clinical features

Most patients with ALPS manifest persistent lymphadenopathy, splenomegaly, or both (Table 2). In the authors' current patient series, the median age at which the initial presentation to a physician occurred is 24 months (range, birth to 15 years). Some patients exhibit splenomegaly without evidence of chronic peripheral lymphadenopathy; a minority has adenopathy without splenomegaly.

The degree of lymphadenopathy is variable. At one end of the spectrum are patients with massive lymphadenopathy that distorts normal anatomic landmarks and can be both palpated and seen, termed *4+ adenopathy*. In other patients, the degree of lymph node enlargement is more modest and is within normal limits for age. By CT, enlargement of abdominal and thoracic lymph nodes is also frequently seen [7].

Table 1. Criteria for the diagnosis of autoimmune lymphoproliferative syndrome

Required features
Chronic nonmalignant lymphadenopathy, splenomegaly, or both
Increase* in circulating T cells that are $CD4^-CD8^-$ and express the α/β T-cell receptor
Demonstration of defective antigen-induced lymphocyte apoptosis on *in vitro* culture
Supporting features
Family history of autoimmune lymphoproliferative syndrome
Typical findings on histopathologic analysis of lymph node or splenic tissue
Autoimmune disease
Mutations of genes encoding Fas or related apoptosis signaling proteins

*More than 1% of peripheral blood lymphocytes.

Table 2. Clinical features in 79 patients with autoimmune lymphoproliferative syndrome*

Sex	
Female	43
Male	36
Race	
White	69
Black	6
Other	4
Median age at presentation (range)	2 y (birth–15 y)
Manifestation	Patients, n (%)
Lymphoproliferative disease	79 (100)
Splenomegaly	75 (95)
Lymphadenopathy	76 (96)
Hepatomegaly	57 (72)
Lymphoma	7 (9)
Autoantibodies†	64 (81)
Anticardiolipin antibody	51 (65)
Positive direct Coombs test	40 (51)
Antinuclear antibody	20 (25)
Rheumatoid factor	11 (14)
Anti-Factor VIII	1 (1)
Autoimmune disease	37 (47)
Hemolytic anemia‡	23 (29)
Idiopathic thrombocytopenic purpura	18 (23)
Neutropenia	15 (19)
Glomerulonephritis	2 (3)
Optic neuritis or uveitis	2 (3)
Guillian-Barré syndrome	1 (1)
Primary biliary cirrhosis	1 (1)
Coagulopathy/Factor VIII inhibitor	1 (1)

*Data compiled from probands and relatives with autoimmune lymphoproliferative syndrome (47 discrete families).
†Thirty patients had one or more autoantibodies but no autoimmunity disease.
‡Direct Coombs test was positive for both IgG and C3d in all patients with hemolytic anemia.

The lymph nodes in ALPS reveal architectural preservation with florid reactive follicular hyperplasia and marked paracortical expansion with immunoblasts and plasma cells [8]. The paracortical expansion may be extensive enough to consider a differential diagnosis of immunoblastic lymphoma, and although lymphoma is prevalent in ALPS, the overall stability of the clinical picture over a period of many years argues strongly against lymphoma. Increased numbers of $CD3^+$ T cells that are also $CD4^-CD8^-$ are detected in the paracortical region of lymph node tissue. The splenic tissue reveals lymphoid hyperplasia of the white pulp with histologic features similar to those of the lymph nodes. This combination of follicular hyperplasia and paracortical expansion by a mixed infiltrate containing $CD4^-CD8^-$ T cells appears to be unique to ALPS and helps differentiate this syndrome from other benign and malignant lymphoproliferative lesions.

Circulating autoantibodies, overt autoimmune disease, or both are found in the majority of cases. Potentially pathogenic autoantibodies are seen in the absence of overt autoimmune disease. The most common autoantibodies detected in ALPS are directed against red blood cells and detected by the direct Coombs test. Anticardiolipin antibodies are also frequently seen, but among 51 sero-

positive patients with ALPS, the authors have entertained the possibility of a thrombotic or embolic event in only one.

The most common autoimmune diseases seen in ALPS are hemolytic anemia and idiopathic thrombocytopenic purpura (ITP). Hemolytic anemia is associated with IgG autoantibodies to red blood cells and is frequently severe. Likewise, ITP is usually severe, with platelet counts frequently falling below 20×10^9/L. It is not uncommon for patients to exhibit both autoimmune hemolysis and ITP, either concomitantly or as separate events, an entity long termed *Evan syndrome*.

Neutropenia (absolute count < 1.0×10^6 cells/L) is another frequent finding in ALPS. It appears to result from autoimmune mechanisms because it often develops after splenectomy and in the setting of normal myeloid cellularity on bone marrow examination. In addition, the authors have seen neutrophil counts normalize in several patients during glucocorticoid and cytotoxic therapies.

Several nonhematologic autoimmune diseases have also occurred in patients with ALPS. In the authors' early series, one patient developed a severe inflammatory polyneuropathy consistent with Guillain-Barré syndrome. Glomerulonephritis developed in two patients, in one of whom it was associated with a positive antinuclear antibody test. Several years after the glomerulonephritis resolved, this same patient developed ITP and autoimmune liver disease that resembled primary biliary cirrhosis. Antinuclear antibodies were not observed in the second patient with glomerulonephritis; however, this patient also had episodes of both ITP and autoimmune hemolytic anemia. Other autoimmune conditions observed include coagulopathy associated with anti-Factor VIII antibodies, acute disseminated encephalomyelitis, and other conditions. In ALPS there is the potential for multiple autoimmune diseases involving different organ systems to occur in a single patient.

Immunologic studies

The most prominent immunologic abnormalities include a T-cell and B-cell lymphocytosis, increased numbers of circulating $CD4^-CD8^-$ double-negative T lymphocytes that express the α/β T-cell receptor, and a polyclonal hypergammaglobulinemia. The magnitude of these abnormalities is variable. Patients with the most severe lymphoid hyperplasia tend to exhibit the most pronounced lymphocytosis, the largest numbers of circulating $CD4^-CD8^-$ T lymphocytes, and the highest serum immunoglobulin levels.

The origin of the double-negative T cells is unclear. Functionally, they are relatively anergic, exhibiting a decreased capacity to proliferate and secrete cytokines in response to a variety of *in vitro* stimuli [3,9,10•]. Phenotypically they express a pattern of surface markers that suggests that they are derived from cytotoxic $CD8^+$ T cells [8,10•,11•]. Patients with ALPS also manifest increased numbers of circulating $CD4^+$HLA-DR^+ cells that appear to be activated T cells. When stimulated *in vitro*, these cells exhibit a TH2 pattern of cytokine production [9,10•]. These findings raise the possibility that a skewed TH2 response by chronically activated CD4 T cells may play a role in the pathogenesis of autoimmune disease in ALPS.

As indicated, all patients with ALPS by definition exhibit defective lymphocyte apoptosis. Testing for defective lymphocyte apoptosis requires specialized assays that are not commercially available [4]. Briefly, one cultures lymphocytes from patients and controls in the presence of interleukin-2 for several days, stimulates them with phytohemagglutinin, and then triggers apoptosis with a monoclonal antibody that binds to and activates Fas. The majority of normal cells are killed, whereas the minority of cells from patients with clinical and immunologic features of ALPS undergo apoptosis.

Molecular genetic and family studies

The majority of ALPS cases are associated with heterozygous mutations in the Tumor Necrosis Factor Receptor SuperFamily-6 (TNFRSF6) gene encoding Fas that are inherited in an autosomal dominant fashion. *In vitro* studies showed that the mutant Fas protein inhibits the function of normal Fas proteins [4]. This explains how heterozygous Fas mutations behave in an autosomal dominant manner.

Detailed analyses of extended pedigrees of patients with ALPS with TNFRSF6 mutations identified not only a carrier parent but also other family members with identical heterozygous mutations. Although family members with TNFRSF6 mutations exhibited defective *in vitro* lymphocyte apoptosis, most of them had few, if any, clinical or immunologic features of ALPS. Detailed molecular analyses revealed that mutations involving the intracellular domain of the Fas molecule are more likely to be associated with clinical or immunologic features of ALPS than were mutations affecting the extracellular domain [12].

With detailed molecular genetic analysis of a large number of ALPS kindreds, it became apparent that a minority of cases were not associated with mutations in the TNFRSF6 gene. A single patient with ALPS and a mutation in the gene encoding Fas ligand has been described [13]. In addition, mutations in the gene encoding caspase-10 (an enzyme in the Fas-apoptosis pathway) have been reported in two patients with ALPS [14]. These findings have led the authors to propose a classification scheme for ALPS that is based on the site of the

mutation. People with ALPS *type Ia* have mutations in the TNFRSF6 gene. People with mutations in the Fas ligand gene are referred to as *type Ib*, and those with caspase-10 mutations are referred to as *type II*. People with ALPS and uncharacterized defects in the apoptotic pathway are designated as *type III*.

Recently, a unique syndrome sharing certain features with ALPS has been reported in a single kindred with inherited genetic deficiency of caspase-8 [15••]. Affected people exhibited chronic lymphadenopathy, splenomegaly, and defective Fas-mediated lymphocyte apoptosis. Unlike people with ALPS, these patients suffered from recurrent herpes simplex virus and bacterial sinopulmonary infections and had poor responses to immunization. *In vitro*, the affected people exhibited activation defects in T lymphocytes, B lymphocytes, and natural killer cells.

Treatment

With the exception of performing splenectomy for severe and refractory hypersplenism, the authors generally do not direct treatment at the lymphoproliferative process itself. However, in patients who received prolonged, high-dose glucocorticoid therapy (≥1 mg/kg/d prednisone) or other immunosuppressive drugs for treatment of autoimmune disease, the size of individual lymph nodes may decrease transiently but returns to the original size after tapering or termination. The preference is to avoid splenectomy. To reduce risks of splenic rupture, the authors custom-fit children with ALPS with a fiberglass abdominal guard. Aggressive contact sports are strongly discouraged.

When splenectomy proves unavoidable, the authors provide conjugate and polysaccharide vaccines for *Streptococcus pneumonia*, *Hemophilus influenza* type B, and *Neisseria meningitidis*, according to published recommendations. Because of what appears to be a higher than expected rate of postsplenectomy pneumococcal sepsis in patients with ALPS, the authors monitor antipneumococcal titers in these patients. If titers fall below protective levels (and the authors' data suggest that titers are not maintained as well as one would hope in the setting of ALPS), booster doses of the conjugate pneumococcal vaccine are administered. Antibiotic prophylaxis is provided into later adolescence and continued in adults with previous episodes of pneumococcal bacteremia.

A recent study from Belgium examined the use of sulfadoxine/pyrimethamine (Fansidar; Roberts Pharmaceuticals, Eatontown, NJ) in the treatment of seven patients with ALPS [16]. Only two of these seven patients had TNFRSF6 mutations, and in two patients, lymphocyte apoptosis was normal. Positive clinical responses were claimed to have occurred in all seven patients after sulfadoxine/pyrimethamine therapy. However, detailed data on the magnitude of these responses were not provided. Given the severe hypersensitivity reactions that have been associated with sulfadoxine/pyrimethamine [17,18], the routine use of this drug to treat patients with ALPS cannot be recommended until more data on efficacy become available.

The treatment of ITP, autoimmune hemolytic anemia and all autoimmune manifestations are the same as in patients without ALPS. As indicated, episodes of autoimmune hemolytic anemia and ITP are frequently severe and usually require treatment with high doses of glucocorticoid (≥ 1 mg/kg/d prednisone). In some cases, it is possible to taper and discontinue glucocorticoid therapy over a period of 10 to 14 days. In others, tapering leads to prompt relapses and the need for extended glucocorticoid therapy at the lowest possible daily or alternate day dosing, the use of glucocorticoid-sparing agents, or both. For all patients on prolonged (>3 months) glucocorticoid therapy, the authors provide calcium and vitamin D supplementation. In adults, the authors add bisphosphonate drugs to help prevent glucocorticoid-induced osteoporosis.

In the authors' experience, treatment of ITP with high-dose intravenous immunoglobulin was usually either ineffective or produced only transient increases in platelet counts. Several of the authors' patients with ALPS and ITP have required the addition of cytotoxic agents such as azathioprine or mycophenolate mofetil to maintain platelet counts above 20×10^9/L. There is a recent report of a single patient with ALPS with refractory ITP responding to treatment with both rituximab and vincristine [19]. Thrice weekly to daily injections with low doses (1–2 μg/kg) of recombinant granulocyte colony-stimulating factor has been effective at producing sustained increases in neutrophil counts in a small number of patients with ALPS with neutropenia and recurrent infections.

Prognosis

Because ALPS was recognized only recently as a clinical entity, most patients have been followed for a relatively short time. Thus, the authors are not certain of its long-term prognosis. So far, only three of the authors' cohort of 79 patients with a confirmed diagnosis of ALPS have died of causes directly related to this syndrome. There had been several deaths in the authors' study families before ALPS had been diagnosed. The major determinants of morbidity and mortality in ALPS are the severity of autoimmune disease, postsplenectomy sepsis, and lymphoma.

Some information on long-term prognosis of ALPS can be gained from studies of the authors' patients who are now adults. Based on old medical records, some of these patients clearly had manifestations of ALPS in early childhood. For example, one patient has persistent

lymphadenopathy but is otherwise well at age 30 years. A second patient had the onset of lymphoproliferative disease in early infancy and had a splenectomy at age 2 years. This patient's peripheral lymphadenopathy resolved during adolescence, but she developed ITP at age 18 years and autoimmune neutropenia at age 32 years. Thus, patients with ALPS may be at lifelong risk for the development of autoimmune disease. A third adult patient has persistent lymphadenopathy and developed Hodgkin disease at age 26 years. This case led the authors to investigate the incidence of lymphoma in 130 members of 39 families with FNFRSF6 mutations [20]. Eleven B-cell and T-cell lymphomas of diverse types developed in 10 people. This result represents a risk of non-Hodgkin and Hodgkin lymphomas, respectively, that is 14 and 51 times greater than expected. The ideal prospective treatment of patients with chronic lymphadenopathy and splenomegaly who are at increased risk of lymphoma has still not been defined.

Conclusions

Autoimmune lymphoproliferative syndrome is a newly recognized clinical syndrome associated with abnormal lymphocyte apoptosis. The lymphoproliferative disease presents in childhood and has unique immunologic and pathologic features that allow it to be differentiated from other benign and malignant conditions. Autoimmune disease occurs in many patients at some time. The short-term prognosis of patients with ALPS appears good. However, these patients are at increased risk for the development of malignant lymphoma.

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