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Autoimmune lymphoproliferative syndrome

A genetic disorder of abnormal lymphocyte apoptosis

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As genetic accidents of nature, primary immunodeficiency disorders have furthered the understanding of the human immune system. Likewise, a recently recognized human genetic disorder of abnormal lymphocyte survival, autoimmune lymphoproliferative syndrome (ALPS), has illustrated the importance of a carefully maintained homeostatic balance between lymphocyte survival and lymphocyte death by a process called apoptosis (or programmed cell death) [1]. At the most basic level, ALPS is a disorder of defective lymphocyte apoptosis that involves an antigen-driven pathway of cellular suicide, in which apoptosis is induced by the interaction of the cell-surface receptor Fas (CD95) with its ligand, Fas ligand (FasL). Most patients with ALPS have a defect in the gene for Fas, the sixth member of the tumor necrosis factor receptor superfamily (*TNFRSF6*). In several other patients, defects in the gene for FasL (*TNFSF6*), or down-stream proteins of the Fas pathway (caspase 10), have been identified [2,3]. An increasing number of patients with ALPS are being recognized, with possible genetic defects that remain to be identified.

From a historical perspective, early clues to the nature of this disorder were provided by the recognition that certain clinical and laboratory features of patients with ALPS were shared by two well-characterized animal models of human autoimmune disease: (1) the MRL/Mp-*lpr/lpr* and (2) C3H/HeJ-*gld/gld* mouse strains (referred to as *lpr* and *gld*, respectively) [4–6]. These mice subsequently were found to have defective Fas-mediated apoptosis, secondary to genetic defects in Fas (*lpr*) and FasL (*gld*) [5,6]. Subsequent evaluation of patients with ALPS revealed a similar apoptotic defect and genetic mutations in *TNFRSF6* [8,9].

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Criteria, classification, and epidemiologic features

Criteria that are used by the National Institutes of Health (NIH) ALPS Group (NIH Group) to identify patients include the presence of chronic accumulation of nonmalignant lymphoid cells, manifested as lymphadenopathy or (hepato)splenomegaly; defective in vitro Fas-mediated lymphocyte apoptosis; and presence of 1% or more T cells that express the α/β -T-cell receptor (α/β -TcR) but lack co-expression of CD4 or CD8 on their surface (α/β -double negative T cells [α/β -DNT cells]) in peripheral blood, or the presence of DNT cells in lymphoid tissue (required criteria) [10]. Supporting criteria include presence of autoimmunity or autoantibodies and a family history of ALPS [10]. Based on the genetic defect, ALPS is classified as follows: ALPS type Ia, caused by a mutation in *TNFRSF6*; ALPS Ib, caused by a mutation in *TNFSF6*; ALPS type II, caused by a mutation in the gene for cystinyl-aspartate-requiring-proteinase (caspase) 10 (*CASP10*); and ALPS type III, which is the provisional diagnosis when ALPS types Ia, Ib, and II have been ruled out. ALPS is a rare condition with no current estimate of disease incidence. It has been observed in diverse ethnic populations and occurs in both sexes. A skewing toward men has been noted in the NIH ALPS cohort, contrasting with the predominance of affected women in many other autoimmune diseases.

Pathogenic and genetic considerations

Lymphocytes, like all lineages of blood cells and other cells, rely on genetically determined programs of cellular suicide (apoptosis) to preserve their homeostatic balance. Many of these programs use common intracellular molecules. An important aspect of apoptosis is that the execution of the apoptotic program and subsequent removal of apoptotic cells generally does not induce an inflammatory response, in contrast to the potentially harmful inflammatory response to acute nonphysiologic cell injury and subsequent necrosis [11].

The Fas pathway of apoptosis is positioned at an important crossroad of T-lymphocyte homeostasis (reviewed in reference [1]). After the induction of antigen-specific immune responses, Fas-mediated apoptosis, through suicide and fratricide (apoptosis of neighboring T cells), contributes to the down-regulation of the immune response, including an immune response to self-antigens. Through apoptosis, clonotypic diversity and, perhaps, immune-privileged sites are maintained [12].

Cell-surface expression of Fas is linked to T-cell activation. Following activation and clonal expansion, T cells express high levels of Fas and become susceptible to Fas-mediated apoptosis. An additional requirement for T-cell apoptosis is that the activated T cells receive a “competency to die” signal provided by restimulation through their TcRs [13]. This step is an important safeguard to ensure that only the responding antigen-specific T cells are subjected to Fas-mediated apoptosis. Fas-induced apoptosis involves interaction of Fas with

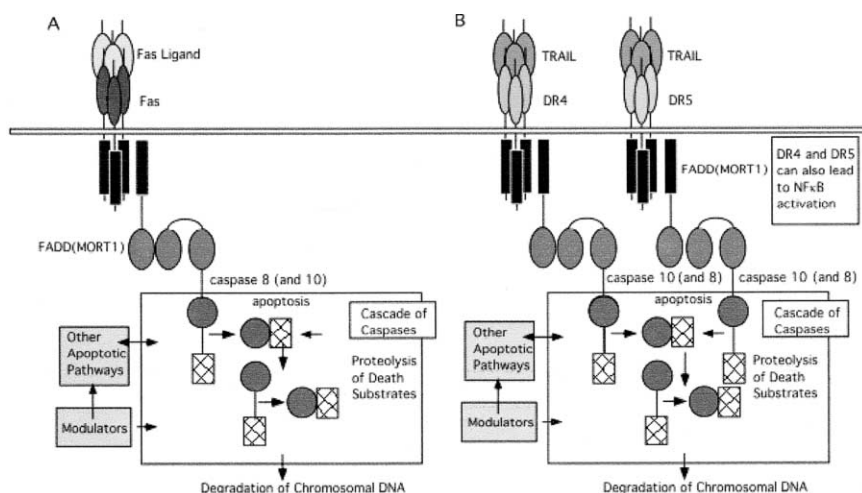


Fig. 1. Apoptosis pathways. (A) Trimeric Fas binds to trimeric FasL following an immune response. Intracellularly, the death domains of Fas (*rectangle*) associate with FADD (mediator of receptor-induced toxicity [MORT1]), which then binds caspase 8 and caspase 10. This action initiates the proteolytic caspase cascade, leading to degradation of chromosomal DNA and cell death. (B) Cell death also can be initiated by other members of the TNFSF family, such as TNF and TRAIL that interact with members of the TNFRSF family, such as TNFR1, DR3, DR4 (TRAIL-R1), and DR5 (TRAIL-R2) that harbor cytoplasmic death domains. This pathway seems to prefer interaction with caspase 10. DR4 and DR5 also can engage NFκB. (From Autoimmune lymphoproliferative syndrome database (ALPSbase). Available at: http://www.nhgri.nih.gov/DIR/GMBB/ALPS/alps_molecular_pathways.html. Accessed April 2002.)

FasL, expressed on other T cells and with soluble FasL and released from T cells, and requires that FAS and FASL form a homotrimer (Fig. 1). Following FasL engagement, intracellular recruitment of the cytoplasmic adaptor protein FADD (Fas-associated protein with death domain) to the trimerized cytoplasmic death domains of Fas takes place (Fig. 2). Subsequently, the cytosolic molecules procaspase 8 and procaspase 10 bind by way of their death effector domains (DEDs) to a similar DED on FADD. Together, Fas, FADD, and the caspase molecules form the death-inducing signaling complex (DISC). Autoprocessing of the inductor procaspase 8 and procaspase 10 into their active caspase forms initiates

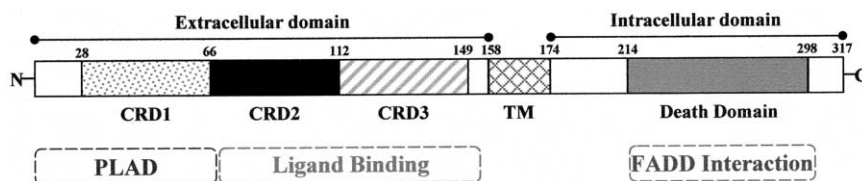


Fig. 2. Organization of the Fas protein. TM = transmembrane.

a lethal proteolytic cascade, involving activation of other effector caspases that cleave specific substrates to generate the changes typical of apoptosis.

In mature lymphocytes, the Fas pathway is an important apoptotic pathway that is responsible for the elimination of infected, self-reactive, and malignant T cells. Death signals can be presented and processed by other pathways that can operate in parallel with the Fas pathway [1,13]. Tumor necrosis factor (TNF) and similar molecules can, like FasL, engage other (trimerized) members of the TNFR superfamily that harbor cytoplasmic death domains, such as TNFR1, death receptor-3 (DR3), DR4 (TNF-related apoptosis-inducing ligand [TRAIL]-R1), and DR5 (TRAIL-R2) [1]. DR4 and DR5 can transduce their signals through NF κ b [14].

Fas is encoded by the gene *TNFRSF6*, which is located on chromosome 10q24.1. The gene structure is as follows: Exons 1 and 2 encode a signal sequence that, on trafficking of the Fas protein to the cell surface, is cleaved off. Exons 3, 4, and 5 encode three extracellular, cysteine-rich domains (CRDs; see Fig. 2). Exon 6 encodes the transmembrane domain. The intracellular domains of Fas are encoded by exons 7 to 9, with exon 9 representing the death domain that interacts with FADD [15]. As of August 2001, more than 60 unique mutations in *TNFRSF6* have been identified (Fig. 3). These mutations are not distributed evenly through-

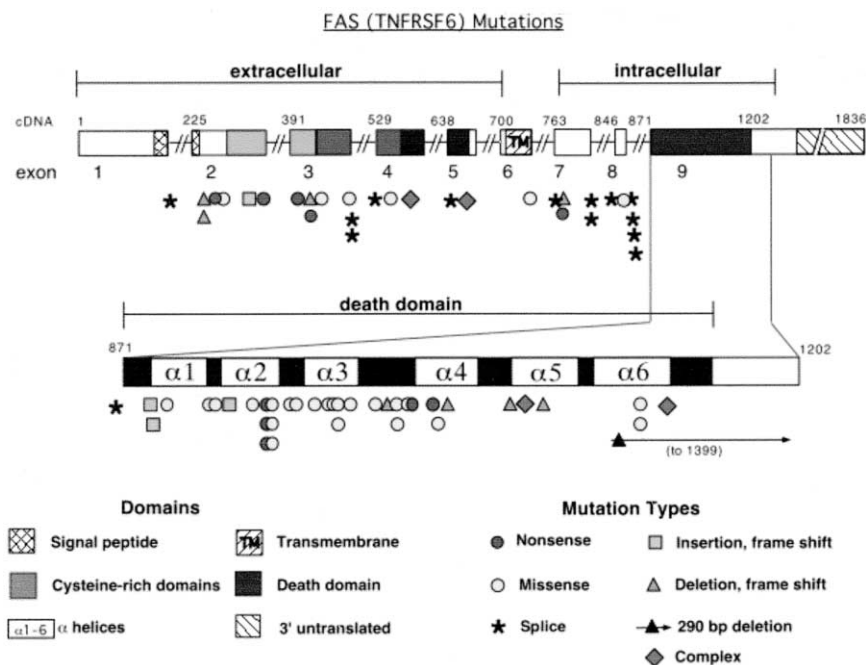


Fig. 3. Organization of the *TNFRSF6* gene. The nine exons of *TNFRSF6* with ALPS-related mutations are shown. Exon 9 is expanded to show the α helical regions of the death domain and the predominance of mutations in the death domain. (From Autoimmune lymphoproliferative syndrome database (ALPSbase). Available at: http://www.nhgri.nih.gov/DIR/GMBB/ALPS/alps_molecular_pathways.html. Accessed April 2002.)

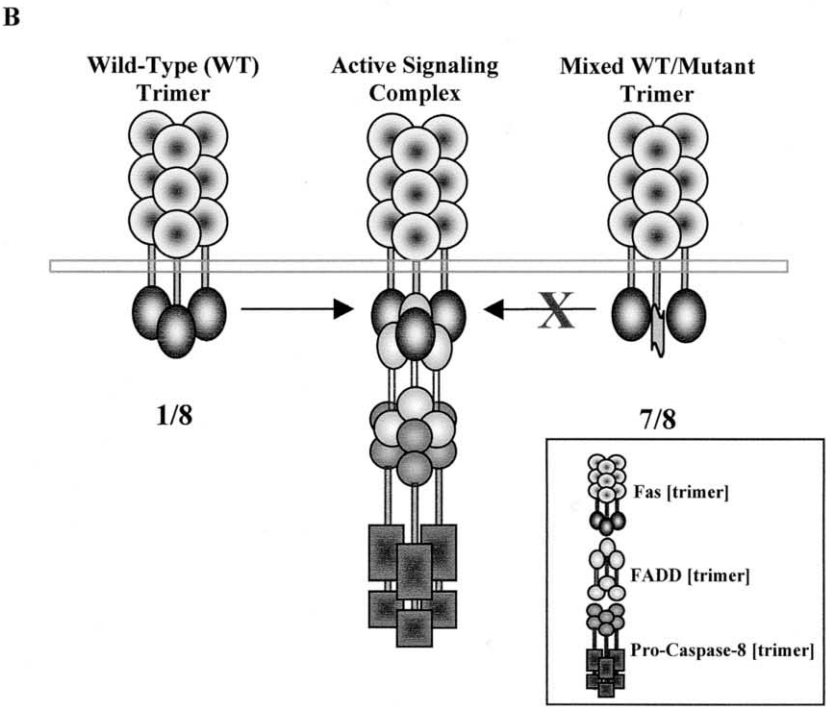
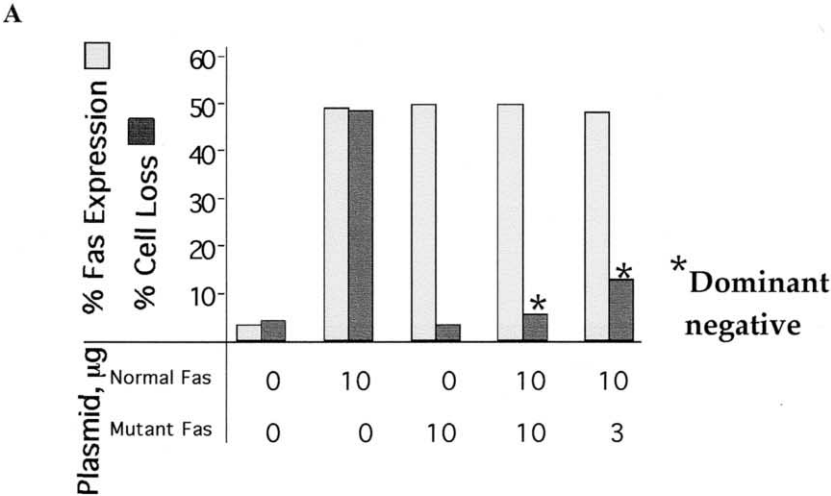
out the gene; most mutations are clustered in exon 9, affecting the death domain. Typically, mutations involve substitutions, additions, or deletions of one or a few nucleotides in the coding regions or the splice sites (see also the ALPSbase at <http://www/nhgri.nih.gov/DIR/GMBB/ALPS>).

In contrast with certain inherited immunodeficiency disorders, such as severe combined immunodeficiency syndrome, *de novo* mutations have been detected in only a few families with ALPS. From a standpoint of population genetics, this finding suggests that ALPS has different consequences for survival and generation of offspring. Genetic analysis has revealed that ALPS is inherited in an autosomal dominant fashion in most families [8]. In three families, autosomal-recessive ALPS is associated with homozygous Fas mutations. In these (consanguineous) families, there was a complete absence of Fas protein and a severe clinical phenotype of prenatal or perinatal onset [9,16,17].

That heterozygous mutations lead to defective, Fas-mediated apoptosis can be explained by a dominant negative effect of the mutated Fas protein. In *in vitro* transfection studies, mutant and wild-type Fas were coexpressed on the surface of a Fas-negative cell line to simulate the heterozygous defect present in patients with ALPS [8]. Functional studies of these cotransfected cells demonstrated that the mutation-bearing Fas proteins interfered with Fas-mediated apoptosis when they were mixed with normal (wild-type) proteins (Fig. 4A). Because Fas and FasL form homotrimers, the contribution of the mutant *TNFRSF6* allele versus the normal *TNFRSF6* allele to these trimers results in a normal Fas trimer in only one out of eight configurations and in an abnormal Fas trimer in seven out of eight possible configurations, assuming equal amounts of mutant and wild-type Fas [8,18]. Dominant negative interference by mutant Fas chains has been demonstrated for all ALPS-associated death domain mutations and for other intracellular mutations and many extracellular mutations [18].

The link between dominant negative interference and defective Fas-mediated apoptosis was strengthened in a study of a series of patients with ALPS and death domain mutations. Localized and more extensive disruptions of the death domain led to an inability to interact with FADD, assemble the DISC, and activate the downstream caspase cascade [19]. That extracellular mutations affecting CRD2 and CRD3 also were associated with a dominant negative effect (as demonstrated by transfection experiments) was more difficult to explain, because these mutations were associated with a lack of binding of anti-Fas antibodies or FasL. Previously, it had been assumed that interaction with trimeric FasL constitutes the required trigger for oligomerization of single Fas chains into trimers [20,21]. In that scenario, mutations in the ligand-binding domain (CDR2 and CRD3) would interfere with FasL binding, prevent trimerization of any Fas protein that contained mutant chains (seven out of eight), and preclude dominant interference. This apparent dilemma was solved when it was recognized that functional Fas receptors, like other TNFRSF members, self-associate into homotrimers before interaction with (trimeric) FasL [22,23]. This preassociation of Fas is mediated by the NH₂-terminus and the CRD1 domain, referred to as the preligand assembly domain (see Fig. 2). Even

in the presence of extracellular mutations that affect the ligand-binding domain, faulty Fas trimers (seven out of eight) are assembled that dominantly interfere with Fas-mediated apoptosis.



For other extracellular mutations, including mutations that might affect the function of the preligand assembly domain, defective apoptosis can be explained through interference by truncated and soluble fragments of mutant Fas that interact with FasL, or by haplo-insufficiency in which the total amount of generated Fas is below a threshold that is needed for physiologic induction of apoptosis. In homozygous and compound heterozygous mutations, in which patients have inherited a (different) Fas mutation from both parents, defective Fas-mediated apoptosis can be explained by the principle of loss of function.

Much has been learned about the complex interplay between genotype, phenotype, and disease penetrance through the study of family members of patients with ALPS [18,24,25]. These studies confirmed that all *TNFRSF6*-mutation bearing relatives have defective Fas-mediated apoptosis, even if they lack other ALPS features. Defective apoptosis is inherited in an autosomal dominant manner with full penetrance [18,26]. In contrast, clinical and laboratory features have a highly variable expression, as demonstrated by many mutation-positive relatives who lack some or all manifestations of ALPS. Further studies have shown that penetrance of ALPS features, other than impaired apoptosis, is significantly higher in families with intracellular *TNFRSF6* mutations than in those with mutations affecting the extracellular domains [18,24,25]. Penetrance of significant ALPS-related morbidity (splenomegaly requiring splenectomy, autoimmune disease requiring treatment, lymphoma) was also significantly higher in relatives with intracellular mutations [18].

The fact that, in families with ALPS, there are many mutation-carrying relatives who completely lack clinical disease indicates that factors other than Fas mutations are required for the pathogenesis of ALPS. Similar to *lpr*- and *gld*-based mouse models, the genetic background might determine the penetrance of ALPS manifestations, age of onset, or severity of these manifestations. The genetic background might concern detrimental or protective modifications of genes that interact directly with the Fas–FasL pathway. Alternatively, genes relevant to Fas-independent apoptosis pathways might be affected. The genetic background might operate at the level of genes that encode regulators of other aspects of lymphocyte function and survival. Several examples of this last possibility are discussed later in this article. The identification of single nucleotide polymorphisms in the human genome, and the appreciation that these polymorphisms can contribute to human diseases, has opened new avenues for investigation in ALPS and in other immunologic disorders in which genetic backgrounds might have a role [27]. At present, the genetics and pathogenesis of

Fig. 4. Dominant negative interference by mutant Fas protein. (A) Transfection experiments using normal (wild-type) or mutant cDNA from a patient with ALPS. As a target, a murine thymoma cell line that does not express Fas was used. Following transfection, cell-surface expression of Fas was determined by flow cytometry and induction of apoptosis using an antibody to human Fas was evaluated. (B) Dominant negative interference. Assuming equal contribution of normal Fas and Mutant Fas to the trimer, an abnormal trimer is formed in seven out of eight possible configurations, and a normal trimer in only one out of eight possible configurations.

ALPS type Ia can be summarized as follows: Inherited heterozygous mutations in *TNFRSF6*, through dominant negative interference, cause defective Fas-mediated apoptosis that, in combination with additional factors, lead to clinical ALPS, particularly if these mutations affect the intracellular domains of Fas. The same complex relationship between the genetic defect and the clinical phenotype is also evident in two families with ALPS type II (*CASP10* mutations) and likely is present in families with ALPS type III [3].



Fig. 5. Patient with ALPS with extensive lymphadenopathy.

Clinical manifestations

The importance of Fas in the maintenance of lymphocyte homeostasis is reflected in three main consequences (as clinically experienced by patients with ALPS): (1) abnormal accumulation of lymphocytes beyond the containment capacity of the lymphoid system (Fig. 5); (2) autoimmunity caused by a failure to remove autoreactive lymphocytes; and (3) increased occurrence of lymphomas caused by inappropriate survival of transformed lymphocytes (Table 1).

With regard to lymphoproliferation, all patients with ALPS develop lymphadenopathy or splenomegaly during the course of their disease, whereas approximately 50% of patients also exhibit hepatomegaly. The extent and severity of these manifestations varies from patient to patient [10]. In many patients, these manifestations present in the first 2 years of life; in the remainder of patients, the features are usually present before the fifth year of life, although no strict age limit seems to be present. Lymphadenopathy often involves the cervical and axillary chains but can be found at any anatomic location [28]. Important in the differential diagnosis of generalized lymphadenopathy, constitutional signs, such as weight loss, fever, and night sweats, are uncommonly reported in ALPS.

Table 1
Clinical manifestations

Manifestations	Prevalence (%) ^a
Lymphoproliferation of nonmalignant lymphoid cells	100
Lymphadenopathy	87
Splenomegaly (± hypersplenism)	90
Splenectomy	32
Hepatomegaly	45
Autoimmunity	
Autoimmune hemolytic anemia	53
Autoimmune thrombocytopenia	44
Autoimmune neutropenia	31
Glomerulonephritis	8
Autoimmune hepatitis	6
Guillain-Barré syndrome	5
Uveitis, iridocyclitis	5
Neoplasia (including benign tumors) ^b	
Lymphoma (Hodgkin's and non-Hodgkin's lymphoma)	9
Carcinoma (thyroid, breast, skin, tongue, liver)	6
Multiple neoplastic lesions (thyroid and breast adenomas, gliomas)	1
Other findings ^b	
Urticaria and other skin rashes ^c	—
Vasculitis, panniculitis, arthritis, arthralgia, recurrent oral ulcers	1/1/4/1/1
Aplastic anemia, pulmonary infiltrates, premature ovarian failure	1/2/1
Hydrops fetalis	2
Organic brain syndrome (mental status changes, seizures, headaches)	1

^a Data compiled from reported index cases and from unpublished results of index cases studied by the NIH Group.

^b Reported index cases and affected *TNFRSF6* mutation-positive relatives.

^c Present in most patients.

Splenomegaly frequently is associated with hypersplenism, which can be difficult to distinguish from autoimmune cytopenias. Hypersplenism, uncontrollable thrombocytopenia and anemia, traumatic rupture, suspicion of malignancy, and discomfort have been common indications for splenectomy. Because of increasing experience with patients with this disorder, it has been recognized that lymphadenopathy, often massive during childhood, tends to regress during adolescence and adulthood. This finding is less often the case for splenomegaly, which explains the observation of isolated splenomegaly presenting in adult patients. Rare cases of homozygous or compound heterozygous Fas mutations have presented with manifestations of severe lymphoproliferation at or before birth [9,16,17,29].

Autoimmunity associated with ALPS falls roughly into two groups: (1) autoimmunity against blood cells and (2) a group of less frequent autoimmune features [10]. Most patients develop Coombs positive hemolytic anemia or a combination of anemia and autoimmune thrombocytopenia (and less frequently autoimmune neutropenia). This autoimmune profile is a clear distinction from *lpr* and *gld* mice that uniformly develop glomerulonephritis (rare in patients with ALPS) but infrequently develop cytopenias [30]. In contrast to the thrombocytopenia of childhood idiopathic thrombocytopenic purpura, thrombocytopenia in ALPS is associated more often with symptomatic bleeding. Autoimmunity in ALPS follows the typical course of exacerbations and remissions. Other, less frequent, (autoimmune) manifestations are listed in Table 1 and include urticaria and other types of skin rashes that are commonly present in patients with ALPS [10]. These manifestations can be present within specific families with ALPS, perhaps reflective of the role of the genetic background, interacting with the defective apoptosis-related genes.

The concern that defective apoptosis can sufficiently shift the balance between proapoptotic and antiapoptotic factors to cause malignant transformation was validated in a study describing the development of lymphomas in families with ALPS [7]. According to this study, patients with ALPS have a 14- and 51-fold increased risk for non-Hodgkin's and Hodgkin's lymphomas, respectively, compared with that in the general population. Most cases of lymphoma, including B-cell and T-cell lymphomas, have been reported in families with *TNFRSF6* mutations that affect the death domain of Fas. Table 1 lists other malignancies and benign tumors. It remains to be seen if there is also a direct relationship between these neoplasms and Fas mutations. An increasing list of lymphoid and non-lymphoid malignancies that exhibit somatic Fas mutations underscores the role of Fas as a tumor suppressor factor (see reference [10] and references therein).

Laboratory findings

In keeping with the clinical manifestations of ALPS, hematologic findings are equally variable in severity and include lymphocytosis, anemia with signs of hemolysis on blood smear, reticulocytosis, and thrombocytopenia [10,25]. The

degree of reticulocytosis and specific blood smear features can help distinguish autoimmune anemia from hypersplenism. Less commonly, neutropenia and eosinophilia, and lymphopenia (not associated with immunosuppressive therapy) are present. Dyserythropoiesis has been reported [31]. Laboratory findings include the following:

Hematology

- Lymphocytosis, lymphopenia (primary or secondary to treatment)
- Anemia (signs of hemolysis or iron deficiency on peripheral blood smear)
- Dyserythropoiesis
- Reticulocytosis
- Thrombocytopenia
- Neutropenia
- Eosinophilia

Chemistry

- Liver function abnormalities (in case of autoimmune or viral hepatitis)
- Proteinuria (in case of glomerulonephritis)
- Elevated levels of vitamin B₁₂

Immunology

- Expansion of α/β -DNT cells
- Expansion of other lymphocyte subsets
 - α/β -DNT cells
 - CD8⁺ T cells
 - CD57⁺ T cells
 - HLA-DR⁺ T cells
 - CD5⁺ B cells
- Decrease in CD4⁺/CD25⁺ T cells
- Decrease in CD27 expression on B cells
- Increased soluble CD27 in serum
- Elevated levels of IL-10 in serum; increased IL-10 mRNA in DNT cells
- Elevated levels of IgG; elevated IgA; elevated, normal, or decreased IgM; elevated IgE
- Autoantibodies (most often positive direct or indirect antiglobulin test, anti-phospholipid antibody, antinuclear antibody, rheumatoid factor)
- Defective receptor-mediated lymphocyte apoptosis
- Other reported findings (increased soluble CD25, CD30, FasL; decreased soluble Fas; decreased in vitro lymphocyte function)

Chemistry profiles are relatively unremarkable. Abnormalities are generally secondary to specific organ involvement, such as liver function abnormalities in the presence of autoimmune hepatitis. A consistent and poorly understood abnormality in patients with ALPS is the highly elevated levels of vitamin B₁₂ (NIH Group, personal communication, 2001).

The presence of α/β -DNT cells in peripheral blood and lymphoid tissue provided the important link to the murine *lpr/gld* models. It constitutes the

signature laboratory abnormality and is part of the diagnostic criteria of ALPS [4,10]. Previous evaluations suggest that these cells are derived from polyclonally activated cytotoxic T cells that have lost CD8 or CD4 expression, although it remains unclear exactly what forms the driving forces behind the expansion of α/β -DNT cells [4,7,32,33,]. Although activated in vivo, α/β -DNT cells from patients with ALPS respond poorly to in vitro stimulation [4,32,34]. α/β -DNT cells show persistent alterations in glycosylation patterns of cell-surface molecules, such as CD43 and CD45. These alterations are associated with the presence of a CD45R glycoform variant that is analogous to the murine B-cell-specific CD45R isoform, B220, a characteristic marker of α/β -DNT cells in *lpr/gld* mice [35]. Other immunophenotypic T-cell abnormalities include an expansion of cytotoxic CD8⁺ T cells that show increased expression of CD57 and HLA-DR and a reduction in T cells that express CD25 (α chain of the interleukin 2 [IL-2] receptor). In a retrospective study of families with ALPS type Ia, it was determined that expansion of CD57⁺ (HLA-DR⁺)/CD8⁺ T cells and the reduction in CD25⁺ T cells were specific for clinical ALPS, because these abnormalities were not present in mutation-positive relatives who lacked clinical disease [36]. The reduction of CD25⁺ T cells was entirely caused by loss of CD25⁺/CD4⁺ T cells and distinguished between mutation-positive relatives with ALPS and mutation-positive relatives without ALPS, who showed comparable degrees of defective in vitro apoptosis [36]. This finding might point toward another pathogenic factor outside the Fas pathway of apoptosis, because it has been found that a CD25⁺/CD4⁺ T-cell subset seems to have an important immunoregulatory role in preventing autoimmunity (see reference [37]). The combined presence of defective apoptosis and T-cell dysregulation, involving immunoregulatory T-cell subsets, might contribute to the development of clinical ALPS in *TNFRSF6*-mutation positive relatives.

Another highly consistent immunophenotypic abnormality in patients with ALPS includes the expansion of B cells that are predominantly CD5⁺. This abnormality might be related to an abnormal activation state of B cells in patients with ALPS. The manifestations of B-cell activation include elevated serum (polyclonal) immunoglobulin levels and the production of autoantibodies, directed against erythrocytes, platelets, and neutrophils and occasionally directed against rheumatoid factor, antinuclear antigens, and other autoantigens [10,38]. Although anecdotal, there are indications for a reduced antibody response to polysaccharide antigens in patients with ALPS (NIH Group, personal communication, 2002) [39].

Alterations in lymphocyte function also are reflected in several cytokine abnormalities, the most striking of which is the highly elevated levels of serum IL-10, associated with high levels of IL-10 mRNA in DNT cells and IL-10 protein in lymph nodes at the site of these DNT cells [34,40]. IL-10 levels correlate with the penetrance of clinical ALPS and could represent another second factor in ALPS pathogenesis, possible through its role as a growth and survival factor for lymphocytes (eg, CD5⁺ B cells).

In keeping with the criteria used by the NIH Group, patients with ALPS have defective Fas-mediated apoptosis of lymphocytes, whereas mutation-negative

relatives have normal apoptosis (see reference [10] and references therein). With regard to ALPS type Ia, individuals from families with intracellular mutations seem to have more severe defects in apoptosis than do individuals from families with extracellular mutations [36]. Apoptosis is not globally defective, as demonstrated by normal in vitro apoptosis induced by antimetabolites and corticosteroids and by increased spontaneous in vitro apoptosis [8,32,40].

The presence of active apoptosis with phagocytosis of apoptotic lymphocytes also has been observed in histologic specimens of lymph nodes and spleen [41]. In general, histopathologic features that are present in lymphoid tissues from patients with ALPS can be distinguished from other causes of lymph node and spleen pathology. The most common finding that seems to correlate with the severity of disease is paracortical hyperplasia involving infiltration of DNT cells in the interfollicular areas [33]. Other features commonly found in lymphoid tissues include follicular hyperplasia, progressive transformation of germinal centers, and plasmacytosis. Together with an overall intact architecture and absence of clonality, these features are distinct from lymphoid malignancies. Histopathologic features present in other tissues include reactive changes in bone marrow secondary to cytopenias, plasmacytosis, and eosinophilia, and liver abnormalities with infiltration of DNT cells, extracellular hematopoiesis, and, in a few instances, hepatitis with or without bile-duct abnormalities and cirrhosis [33,42].

Diagnostic considerations

The diagnostic criteria used by the NIH Group include chronic accumulation of nonmalignant lymphoid cells, defective in vitro Fas-mediated lymphocyte apoptosis, and the presence of 1% or more α/β -DNT cells in peripheral blood or the presence of DNT cells in lymphoid tissue. Because of the observed delay between the onset of lymphoproliferation and autoimmunity, the presence of autoimmunity is not required for the diagnosis. Lymph node biopsies generally are not required to aid in the diagnosis of ALPS, but in cases of suspected malignancy or an atypical presentation, biopsies are indicated.

Following diagnosis, the first step in ALPS classification depends on the identification of the genetic defect, starting with an analysis of *TNFRSF6*. Mutations that globally affect the death domain of Fas, such as caused by γ frame shifts and truncations of the Fas protein, can be assumed to be harmful and interfere with normal function. The situation is different for more subtle mutations, such as point mutations that can be difficult to distinguish from incidental polymorphisms. Before they can be implicated as interfering with Fas-mediated apoptosis, functional demonstration of the dominant negative effect by co-transfection studies (Fig. 4A) can be indicated. Submission of newly diagnosed *TNFRSF6* mutations to the ALPS mutation database (<http://www.nhgri.nih.gov/DIR/GMBB/ALPS>) is recommended to facilitate exchange of information. If no mutation in *TNFRSF6* is identified, further classification involves evaluation of

TNFSF6 and *CASP10*. A patient is diagnosed with ALPS type III if these genes are found to be unaffected.

Treatment strategies and prognosis

Clinical management of ALPS generally is directed at autoimmune manifestations and malignancies. Lymphoproliferation, as manifested by lymphadenopathy and splenomegaly, does not warrant the use of immunosuppressive agents, unless associated with significant obstructive complications or hypersplenism. Cumulative experience in the management of ALPS indicates that, following cessation of immunosuppressive treatment, manifestations of lymphoproliferation tend to reoccur [43]. Hypersplenism is a relatively common complication of ALPS that is often difficult to separate from coexisting autoimmune cytopenias and is a frequent indication for splenectomy.

The most common autoimmune manifestations that require therapeutic intervention are autoimmune anemia and thrombocytopenia. Compared with childhood idiopathic thrombocytopenic purpura, thrombocytopenia in ALPS does not respond as well to intravenous immunoglobulin (IVIg) infusions but typically can be managed with short courses of high-dose corticosteroids. Autoimmune anemia also is responsive to short courses of corticosteroids. Autoimmune features occasionally require more intensive therapy, including prolonged corticosteroid treatment and chemotherapeutic agents (eg, cyclophosphamide, azathioprine, methotrexate). Because of the increased risk for malignancies, chemotherapeutic agents warrant careful use. Pyrimethamine/sulphadoxine, mycophenolate mofetil, and rituximab (a therapeutic monoclonal antibody directed against CD20 on B cells) have been introduced to aid in the management of patients with ALPS. Pyrimethamine and mycophenolate have been shown to reduce lymphoproliferation in patients with ALPS and *lpr* mice, respectively, whereas rituximab can be used in difficult-to-control B-cell expansion, autoimmunity, and B-cell lymphomas [44–47]. Two patients with severe disease, secondary to homozygous mutations, have undergone successful allogeneic bone marrow transplantation [16,48]. Successful treatment of lymphomas suggest that defective Fas-mediated apoptosis does not impair the response to standard chemotherapeutic agents [7,26].

The prognosis with regard to lymphoproliferation is generally favorable, and most patients experience a partial or complete regression of lymphadenopathy and splenomegaly, usually in the second decade of life. With respect to autoimmune disease, it is not clear if autoimmune manifestations likewise regress or if they follow a course of remissions and exacerbations, typical for autoimmune disease. Further follow-up of patients is needed to define the natural history of these manifestations. From the perspective of the laboratory abnormalities, the expansions of lymphocyte subsets, such as α/β -DNT cells and CD5⁺ B cells, and elevated levels of immunoglobulins generally do not show regression [24,36]. The favorable prognosis is offset, however, by the recognition of the increased

risk for lymphoma [7]. This finding mandates long-term follow-up of patients with ALPS and mutation-positive relatives.

Summary

In patients with ALPS, defective homeostasis of lymphocytes is reflected in abnormal accumulation of lymphocytes beyond the containment capacity of the lymphoid system, resulting in lymphadenopathy, hepatosplenomegaly and hypersplenism, failure to remove autoreactive lymphocytes (associated with autoimmunity), and inappropriate survival of lymphocytes leading to malignant transformation. Several of the laboratory findings are unique for ALPS and reflect defective Fas-mediated apoptosis and abnormal immune regulation. Much has been learned about the molecular mechanisms that underlie defective Fas-mediated apoptosis and the complex relationship between genotype, phenotype, and disease penetrance. Family studies strongly suggest the contribution of additional factors to the pathogenesis of ALPS. These factors might include defective immunoregulation by an altered IL-2/IL-2 receptor system, reflected in the specific loss of CD4⁺/CD25⁺ T cells, or by the highly increased IL-10 levels; other factors might be involved equally. Future efforts directed at careful clinical follow-up and basic scientific investigation are needed to increase the understanding of the incidence, natural history, and pathogenesis of ALPS. These efforts also might be beneficial in the understanding of autoimmune disease in general.

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