

Disorders of Apoptosis: Mechanisms for Autoimmunity in Primary Immunodeficiency Diseases

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Received: 15 November 2007 / Accepted: 4 December 2007 / Published online: 12 January 2008
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Abstract A number of primary immunodeficiency diseases represent a paradox of immunodeficiency and autoimmunity. In this minireview, we present basic concepts of apoptosis and disorder of apoptosis as one of the mechanisms to explain such a paradox between immunodeficiency and autoimmunity, which is exemplified by autoimmune lymphoproliferative syndrome (ALPS).

Keywords Autoimmune lymphoproliferative syndrome · caspases · mitochondria · death receptors

Introduction

Apoptosis or programmed cell death is a physiological form of cell death, which plays an important role in cellular homeostasis, embryogenesis, metamorphosis, and removal of mutated or unwanted cells. In the immune system, apoptosis plays an important role in the selection of T cell repertoire, deletion of self-reactive lymphocytes, killing of target cells by cytotoxic T cells and natural killer cells, contraction phase of an effector immune response, and im-

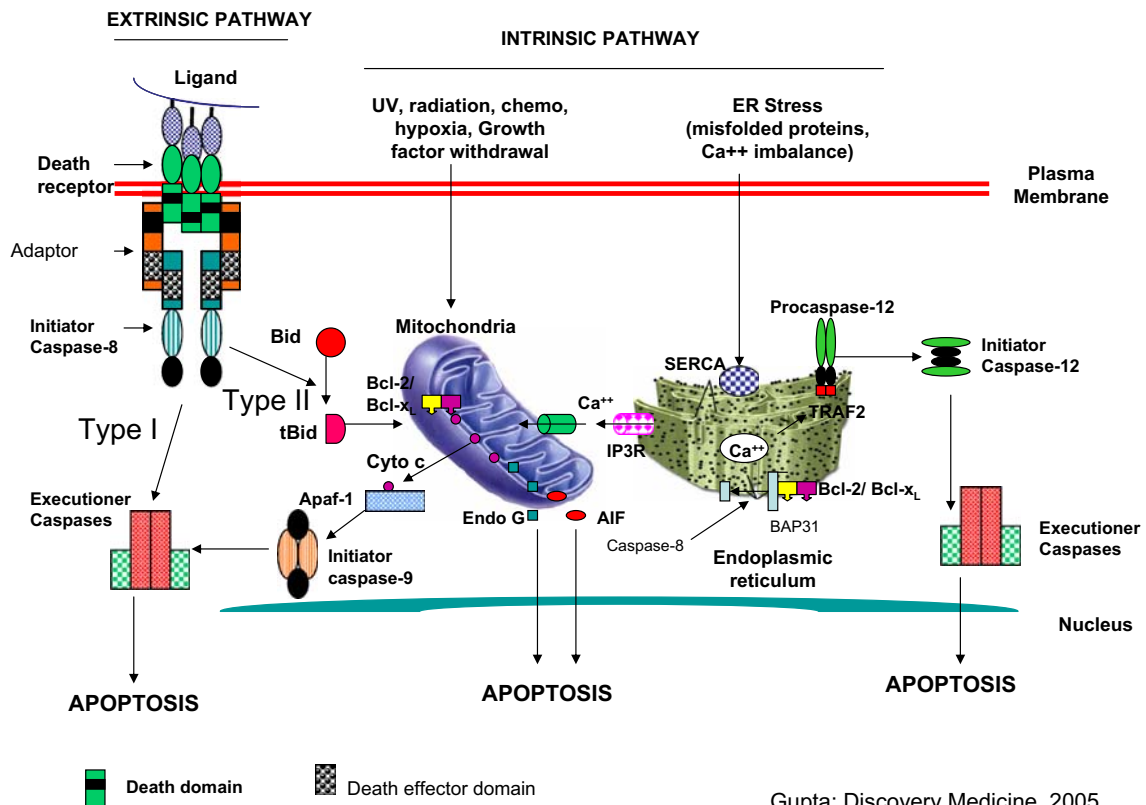
mune privilege. Apoptosis is tightly regulated by a number of highly conserved genes, which either promote or inhibit apoptosis. Apoptosis primarily involves activation of cysteine proteases, the caspase that has affinity to cleave its substrates at a particular aspartate residue. Caspases are present in an inactive prozyme form and are activated by homodimerization either autolytically (as in the case of initiator caspase-8, -10, and -12) or are cleaved to yield active caspases (as in executioner caspase-3, -6, and -7). These caspases act as a molecular chain saw and cleave a number of nuclear, cytoplasmic, and structural substrates, including cell cycle enzymes, DNA repair enzymes, and transcription factors, to induce classical morphological and biochemical features of apoptosis [1, 2]. In addition, in certain cell types and under certain conditions, apoptosis may be mediated by a caspase-independent pathway. Apoptosis is signaled by two major pathways, an extrinsic or death receptor-mediated pathway and an intrinsic pathway, which is mediated via mitochondria and the endoplasmic reticulum (Fig. 1; reviewed in [3–9]). Intrinsic and extrinsic pathways differ in the involvement of distinct initiator caspases and adaptor molecules; however, they involve common executioner caspases.

One of the hallmarks of apoptosis is a lack of inflammation, which is a reason why, when a cell dies by apoptosis, it leaves no trace behind, whereas when a cell dies by necrosis, it is associated with an inflammation. The lack of inflammatory response in apoptosis involves a rapid uptake of apoptotic bodies by the neighboring phagocytes and the induction of intracellular signals resulting in a down-regulation of inflammatory response and induction of anti-inflammatory response, including production of TGF β . Phagocytosis of apoptotic bodies (which contain self antigens, DNA, histones, RNA, RNP, etc.) is facilitated by “eat me” surface molecules, which are recognized by an array of receptors on phagocytic cells (e.g., C1q, phosphatidyl

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Gupta: Discovery Medicine, 2005

Fig. 1 Extrinsic and intrinsic pathways of apoptosis: Extrinsic pathway is mediated by death receptor pathway, whereas intrinsic pathway is mediated via mitochondria and the endoplasmic reticulum

pathway. Different sets of adaptor molecules and initiator caspases are involved; however, all pathways involve common executioner caspases. For details, see the text.

serine and other proteins recognized by CD14, WASP proteins). TGFβ is an important molecule in the generation of regulatory T cells. Therefore, if there is a defect in apoptosis, it may result in both immunodeficiency and autoimmunity. Figure 2 shows the mechanisms for a paradox of autoimmunity in immunodeficiency. An increased apoptosis may result in the depletion of functional lymphocytes

and a defect in uptake of apoptotic bodies (e.g., mutation in C1q associated with SLE; mutation of WASP is associated with autoimmunity in WAS) may lead to (a) late necrosis of apoptotic bodies and the release of self antigens and (b) failure to induce anti-inflammatory response (including production of TGF-β, which is important in the generation of Treg) resulting in immunodeficiency, auto-

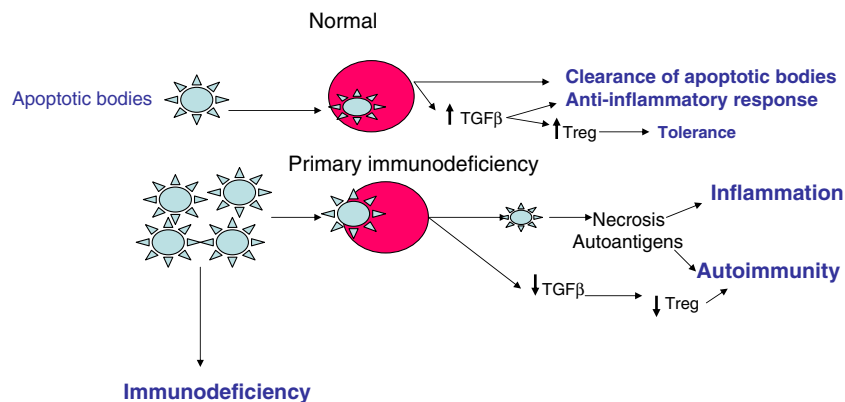


Fig. 2 Proposed model of autoimmunity in primary immunodeficiency: Under normal circumstances, apoptotic cells and bodies are taken up by the neighboring phagocytic cells, resulting in their removal and production of anti-inflammatory cytokines, including TGFβ. TGFβ induces regulatory T cells (*Treg*), resulting in tolerance. In primary immunodeficiency, increased apoptosis and/or defect in the clearance of

apoptotic cells results in decreased production of anti-inflammatory cytokines (including TGFβ) and lysis and necrosis of apoptotic bodies releasing self antigens contained within them. Lysis of apoptotic bodies along with a deficiency of generation of Treg, as a consequence of decreased TGFβ, results in autoimmunity and inflammation.

immunity, and inflammation. In contrast, an inefficient apoptosis may lead to a failure to delete self-reactive lymphocytes and expansion of immature and functionally defective lymphocytes [e.g., autoimmune lymphoproliferative syndrome (ALPS)]. A list of primary immunodeficiencies, which are associated with autoimmunity and disorders of apoptosis, are listed in Table I. We review here the clinical and genetic aspects of the first described human inherited disorder of apoptosis, the ALPS, highlighting the recent advances in the field. In the first part of the review, we briefly recapitulate the molecular pathways governing apoptosis in lymphocytes, which will facilitate the understanding of the genetics and pathophysiology of ALPS.

Death Receptor-mediated Apoptosis

Death receptors belong to a large family of tumor necrosis factor receptors (TNFR), which contain a death domain motif in their cytoplasmic tail. The prototypes and the most-studied death receptors include CD95 and TNFRs.

CD95-mediated Apoptosis

Steps of CD95-mediated apoptosis have been reviewed [1, 5, 9] and are shown in Fig. 3. Ligation of CD95 with CD95 ligand (CD95L) or anti-CD95 antibodies triggers the recruitment of a set of adaptor molecules and procaspases (due to homotypic interactions between their death domain and death effector domain) resulting in the formation of DISC. DISC contains oligomerized/trimerized CD95, Fas-associated death domain (FADD), two isoforms of procaspase-8—procaspase-8a (FLICE or MACH α 1) and procaspase-8b (MACH α 2), procaspase-10, and cellular FLICE inhibitory protein (FLIP). The formation of DISC

results in autoproteolytic activation of initiator caspases, procaspase-8 and procaspase-10. The activation of procaspase-8 is dependent upon its local concentrations (high concentrations favor) for autoproteolytic activation. The homodimers of procaspase-8 have proteolytic activity, and a proteolytic process appears to occur at the DISC by two cleavage events, resulting the generation of an active caspase-8 tetramer, which is subsequently released from the DISC into the cytosol to activate effector procaspases to induce apoptosis. Procaspase-10 forms active heterodimers at the DISC; however, whether caspase-10 can trigger CD95-induced apoptosis in the absence of caspase-8 is controversial; levels of procaspase-10 at the DISC are not sufficient to trigger apoptosis alone [10, 11]. Based on the concentration of caspase-8 at the DISC, CD95-mediated apoptosis signaling pathway is divided into two types [12]. Active caspase-8 concentration at the DISC is high in type I cells. In these cells, active caspase-8 activates effector caspase-3, caspase-6, and caspase-7. In contrast, type II cells are characterized by low levels of active caspase-8 at the DISC and require additional amplifying mechanisms to induce apoptosis. It involves cleavage of BH3-interacting-domain death agonist (BID) by active caspase-8 to generate truncated BID (tBID), which induces aggregation of Bcl-2-associated X protein (Bax) at the mitochondria and release of cytochrome c. In the cytosol, cytochrome c binds to an adapter apoptosis-activating factor [apoptotic protease-activating factor (Apaf-1)] to form a large protein complex, apoptosome, along with procaspase-9. This is followed by activation of procaspase-9 to active caspase-9, which in turn activate effector caspase-3, caspase-6, and caspase-7 to induce apoptosis. Type II signaling is blocked by Bcl-2 and Bcl-x_L, whereas type I signaling cannot be blocked by Bcl-2 or Bcl-x_L [12]. CD95-mediated apoptosis in T cells is predominantly mediated via type I signaling.

Table I Disorders of Apoptosis in Primary Immunodeficiency

Disorder
Increased apoptosis
ADA-deficiency-mitochondrial pathway (p53-mediated)
Cartilage hair hypoplasia syndrome-CD95-mediated
Omenn syndrome-CD95-, TNFR, oxidative stress-mediated
Ataxia telangiectasia-mitochondrial pathway (ATM-p53)
Wiskott–Aldrich syndrome (CD95-, mitochondrial, defect in clearance of apoptotic bodies)
DiGeorge anomaly (CD95-mediated)
Common variable immunodeficiency (TNFR-mediated)
Selective IgA deficiency (BCR-induced caspase-1-mediated)
Kostmann syndrome (<i>HAX1</i> gene mutation)
Decreased apoptosis
Autoimmune lymphoproliferative syndrome mutations of CD95, CD95L, caspase-10, NRAS mutation
Caspase-eight deficiency state mutations of caspase-8

TNFR-mediated Apoptosis

While pleiotropic effects of TNF- α are mediated by binding to type I and type II receptors (TNFR-I and TNFR-II), the death-inducing signal is primarily mediated via TNFR-I. Both cell survival and cell death signals mediated by TNFR require distinct sets of adapters and other downstream signaling molecules. Steps of TNF- α -induced signaling are reviewed [2, 6, 13, 14]. Upon interaction with TNF- α , TNFR-I undergoes trimerization of its receptor death domains, which in turn recruit an adaptor protein, TNFR-associated death domain (TRADD). To induce death signal, TRADD recruits FADD. Therefore, for death-inducing signaling via CD95 or TNFR, FADD serves as a common conduit. The remaining downstream signaling steps are similar to those described above for CD95-mediated apoptosis. Alternatively, for the survival and other biological

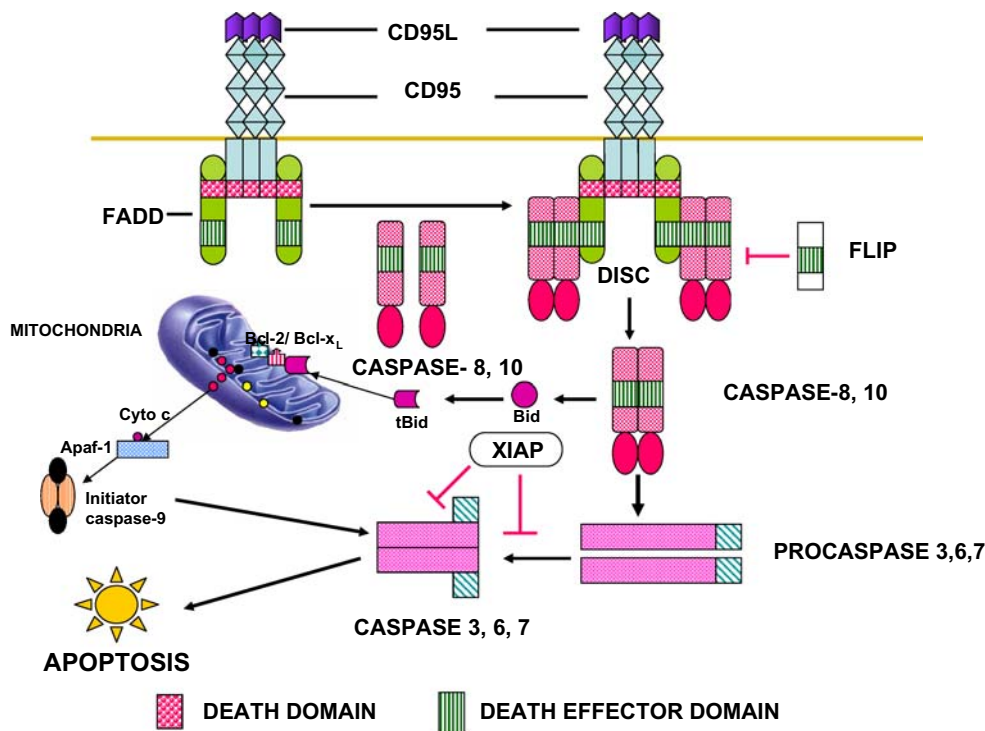


Fig. 3 CD95-mediated apoptosis pathway: Upon ligation with CD95 ligand (*CD95L*), CD95 undergoes oligomerization of its death domain (DD), which recruits an adaptor Fas-associated death domain (*FADD*) and then by homotypic protein–protein interaction between their death effector domain (DED), it recruits initiator procaspases (-8, -10), forming a death-inducing signal complex (*DISC*) as a platform for initiation of apoptosis, procaspase-8 and -10 are activated by homodimerization and active caspase-8 and -10 are released from

the DISC into the cytoplasm, where they cleave executioner caspases to form homodimeric active executioner caspases to induce apoptosis. When caspase-8 at the DISC is low, it cleaves Bid to generate truncated Bid (*tBid*), which is translocated to the mitochondria where it promotes apoptosis by releasing cytochrome c. Cytochrome c binds to Apaf-1 and recruits procaspase-9 to form an apoptosome. Active caspase-9 activates effector caspases, resulting in apoptosis. XIAP inhibits the activation and activity of caspase-3.

functions of TNF- α , TRADD recruits distinct sets of adapter proteins, the TNFR-associated factor-2 (TRAF-2) and receptor interactive protein (RIP). TRAF-2 and RIP stimulate pathways leading to activation of MAP kinase and NF κ B. Both NF- κ B and transient activation of mitogen-activated protein kinases (MAPK) induce survival signals, whereas prolonged activation of MAPK promotes apoptosis. MAPK is a family of proteins, including p38, MAPK, and extracellular signal regulatory kinase 1 and 2 (ERK). The antiapoptotic genes that are upregulated by NF- κ B activation include *cIAP1*, *cIAP2*, *XIAP*, *Gadd45 β* , *Bcl-x_L*, *A20*, *TRAF-1*, *TRAF-2*, and *FLIP*.

Mitochondrial Pathway of Apoptosis

A number of stimuli, including chemotherapeutic agents, UV radiation, stress molecules (reactive oxygen and reactive nitrogen species), and growth factor withdrawal, appear to mediate apoptosis via mitochondrial pathways. Steps for mitochondrial pathway of apoptosis have been recently reviewed [3–5]. The inner membrane (IM) of the mitochondria contains various molecules, including ATP

synthase, electron transport chain, and adenine nucleotide translocator (ANT). Under physiological conditions, these molecules allow the respiratory chain to create an electrochemical gradient (membrane potential). The intermembrane space contains holocytochrome c, certain procaspases, adenylate kinase 2, Endo G, Daiblo/Smac, and apoptosis-inducing factor (AIF). Death-inducing signal via mitochondrial pathway results in the depolarization of IM and the release of these molecules into the cytoplasm. Release of these molecules is under control of a number of proapoptotic and antiapoptotic members of Bcl-2 family of molecules. The Bcl-2 family members are divided into three groups: antiapoptotic (Bcl-2, Bcl-x_L, Mcl-1, Bcl-w, and A1, BOO), proapoptotic “BH3 only” (Bid, Bim, Bik, Bmf, Bad, Hrk, BNIP3, PUMA, NOXA) and proapoptotic “BH-123” (Bax, Bak, and Bok, Bcl-xs) proteins. Bcl-2 and Bcl-x_L are major inhibitors of apoptosis and prevent the release of cytochrome c/AIF from the mitochondrial intermembrane space. In contrast, Bax, Bak, Bad, Bid, and Bim are proapoptotic; Bax and Bak play a major role in the release of cytochrome c.

Once released from the mitochondria, cytochrome c binds to an adapter molecule Apaf-1 in the presence of

ATP/dATP and recruits procaspase 9 to form apoptosome. Procaspase-9 is dimerized and activated without undergoing cleavage, and active caspase-9 activates executioner caspases to orchestrate apoptosis.

There is evidence to suggest that certain molecules present in mitochondrial intermembrane space can promote apoptosis in a caspase-independent manner. AIF is a caspases-independent death effector, which, upon induction of apoptosis, translocates from the intermembrane space of the mitochondria to the nucleus. Once in the nucleus, AIF causes chromatin condensation and large-scale DNA fragmentation to fragments of ~50 kbp. EndoG nuclease is also capable of mediating caspase-independent apoptosis. Endo G, upon its release from mitochondrial intermembrane space, appears to directly mediate nuclear DNA fragmentation.

Endoplasmic Reticulum Stress Pathway

Folding, modification, and sorting of newly synthesized proteins, as well as Ca^{++} storage and signaling, are among the main functions of the endoplasmic reticulum (ER). Disturbance of any of these functions can lead to ER stress, which in turn may induce apoptosis. Steps of the ER stress-induced apoptosis have been reviewed [7–9]. Both overload and depletion of the ER Ca^{++} pool can result in changes in protein folding and in ER stress. Several mechanisms have been proposed for linking ER stress to apoptosis, including direct activation of proteases, kinases, transcription factors, and Bcl-2 family of proteins and their modulators [15]. Prolonged ER stress stimulates the activation of procaspase-12. Procaspase-12 is localized in the ER membrane and is activated and cleaved by m-calpain during ER stress or in response to mobilization of intracellular Ca^{++} stores. Activated caspase-12 then activates effector caspases. There is evidence to suggest that the ER also participates as an intermediary in death-receptor-induced (both CD95 and TNF- α) apoptosis. Furthermore, ER calcium overload results in apoptosis via the mitochondrial pathway. Genetic defects in the extrinsic and, more recently, the mitochondrial pathways have been associated to the clinical manifestations of ALPS.

The Autoimmune Lymphoproliferative Syndrome

Clinical Features

ALPS is characterized by the association of benign lymphocyte accumulation (splenomegaly and lymphadenopathy), autoimmunity, hypergammaglobulinemia, propensity to hematological tumors, and the presence of an unusual peripheral mature T cell population bearing TCR $\alpha\beta$, but lacking CD4 or CD8 expression [named double-negative T cells (DNT)] [16].

Defective lymphocyte apoptosis can be demonstrated in vitro in most patients. Clinical descriptions compatible with ALPS date back to 1967, but Sneller and colleagues from the National Institutes of Health have pointed to the striking similarity of the syndrome to certain phenotypic features found on the *lpr* and *gld* mouse strains, specially the lymphoproliferation and expansion of DNT cells, only in 1992 [17]. Later, in 1995, it was found that both human and mice harbored mutations in the same gene, encoding the FAS death receptor. Based on these manifestations, the NIH has created diagnostic criteria for the diagnosis of ALPS (Table II) [18, 19].

Most patients are diagnosed during infancy or early childhood (median age of 24 months), commonly during work up for unexplained lymphadenopathy, splenomegaly, or autoimmune manifestations. By definition, all patients present soft, painless lymphadenopathy without fever or weight loss. Splenomegaly and liver enlargement are also common. The lymphoid hyperplasia fluctuates over time and tends to spontaneously improve with age. This expansion of the lymphoid organs is caused by massive infiltration by nonmalignant DNT cells [20, 21].

Autoimmune phenomena are very frequent in ALPS. Circulating autoantibodies are seen in up to 81% of ALPS patients, with overt clinical autoimmunity found in 47% of patients. Interestingly, the autoimmune aggression is directed to blood elements almost universally, Coombs' positive hemolytic anemia and immune thrombocytopenia being the most common manifestations. Asymptomatic neutropenia is frequently seen and can be immune-mediated or secondary to hypersplenism. Other autoimmune diseases have only sporadically been reported, such as glomerulonephritis, Guillian–Barré syndrome, linear IgA dermatopathy, anti-factor VIII antibodies, optic neuritis, autoimmune hepatitis, and primary biliary cirrhosis. Curiously, one patient with a systemic lupus erythematosus-like phenotype, including positive ANA and anti-DNA antibodies, was found to

Table II Current NIH criteria for the diagnosis of ALPS

Criteria
Required features
Chronic nonmalignant lymphadenopathy, splenomegaly, or both
More than 1% circulating DNT cells
Demonstration of defective in vitro lymphocyte apoptosis (except ALPS Ib and Im)
Supporting features
Family history of ALPS
Typical findings on histopathologic analysis of lymph node or splenic tissue
Autoimmune disease
Mutation of genes encoding Fas or related apoptosis signaling molecules

harbor a deletion of the gene encoding the CD95 ligand, *TNFSF6* [16, 20].

Laboratory Findings

The laboratory hallmark of ALPS is the presence of an expanded population of mature, rearranged CD3⁺ TCRαβ⁺ CD4⁻ CD8⁻ (DNT) cells thought to be originated from aging peripheral CD4⁺ or CD8⁺ T cells that lost coreceptor expression. These cells represent less than 1% of peripheral lymphocytes in normal individuals but can reach up to 40% in ALPS patients. DNT cells express other markers, such as CD27, CD57, HLA-DR, and CD45R isoform B220 [22, 23]. Their role in the pathogenesis of ALPS is unknown, as they are difficult to grow and unresponsive to stimuli in vitro. However, the recent discovery of patients with clinical disease similar to ALPS harboring somatic Fas mutations in DNT cells and a few other cell clones raises the interesting suggestion that these cells are active players in the disease process [24]. Also, it has been shown that DNT secrete high amounts of IL-10, which might contribute to the polyclonal hypergammaglobulinemia and autoantibody production seen in ALPS [25].

In addition to elevated DNT cells, other immunophenotypic abnormalities include high numbers of CD5⁺ B cells, CD57⁺ CD8⁺ T cells and TCRγδ⁺ lymphocytes. Conversely, there is an unexpected decrease in the numbers of CD4⁺ CD25⁺ cells and CD27⁺ B lymphocytes [23]. NK cells and neutrophils are typically unaltered. An association between high eosinophil counts and higher mortality in ALPS patients has been described recently [26]. The cause of the eosinophilia in this subgroup with poorer outcome is unknown.

Antibodies directed to platelets and neutrophils are found in 46 and 35% of patients, respectively, but have no association with the presence of neutropenia or thrombocytopenia [27]. Anticardiolipin antibodies are also common, but are also not associated with thrombotic phenomena. Antinuclear and anti-DNA antibodies are typically negative.

Histopathological findings on lymph node biopsy are unique to ALPS and help to distinguish this syndrome from other lymphoproliferative conditions. Characteristically, there is follicular hyperplasia and paracortical expansion by infiltrating DNT cells. Cytogenetic and clonality studies are important to help to exclude malignancy [28].

An in vitro lymphocyte apoptosis defect can be demonstrated in most ALPS patients. This test is performed only in specialized centers and consists in the activation of peripheral blood lymphocytes for a few days followed by the treatment with an anti-Fas antibody, which induces the death of cells from normal controls but not ALPS patients. The absence of an apoptotic defect does not exclude ALPS,

as patients with rare mutations in the CD95 ligand, harboring somatic CD95 mutations or with derangements of the intrinsic apoptotic pathway, might have normal Fas-mediated apoptosis [24, 29, 30].

Genetics of ALPS

The vast majority of ALPS patients harbor germline mutations in the gene encoding for the Fas (CD95) death receptor, *TNFRSF6*, inherited in an autosomal dominant fashion. A minority of cases is associated to mutations in other components of the CD95 pathway, namely, Fas ligand (CD95L) and caspase 10. Somatic CD95 mutations found on DNT and a few other cell types have also been described in ALPS patients. Mutations in caspase 8 cause a related, but distinct, syndrome, which is discussed later. More recently, a germline *NRAS* mutation was described in an ALPS patient. This is the first defect affecting the intrinsic pathway of apoptosis reported in ALPS. Table III describes the ALPS classification based on the genetic defect.

ALPS, Type Ia

Out of 240 patients studied by the NIH ALPS group, 148 (62%) have mutations in *TNFRSF6* (*CD95*) gene and are classified as type Ia [18, 19, 31]. This gene is located on chromosome 10q24.1 and consists of nine exons. There are over 70 *CD95* mutations reported to date, most heterozygous, missense, single-nucleotide substitutions, affecting the intracellular death domain encoded by exon 9 (for a full description of the mutations, access <http://research.nhgri.nih.gov/ALPS/>). Homozygous *CD95* mutations have only rarely been reported and are associated with a severe phenotype and clinical manifestations in the neonatal period. These patients are sometimes classified as type 0 [32].

Despite the heterozygosity, the disease is transmitted in an autosomal dominant fashion. This is explained by the structure of the CD95 receptor. Like many other TNF-superfamily receptors, CD95 exists in the cell surface as a preassociated trimer, so that seven out of eight trimers will contain at least one mutant protein, rendering the signaling ineffective. This dominant negative effect has been well-

Table III ALPS classification based on the genetic defects

Classification	Genetic defect
Type Ia	<i>FAS</i> (<i>TNFRSF6</i>)
Type Ib	<i>FASLG</i> (<i>TNFSF6</i>)
Type Im	Somatic <i>FAS</i> mutations
Type II	<i>CASP10</i>
Type III	Unknown mutation
Type IV	<i>NRAS</i> (intrinsic apoptosis defect)

demonstrated in vitro by cotransfection of the normal and mutant alleles in cell lines [33].

There is a complex genotype–phenotype relationship in ALPS Ia. Intracellular death domain mutations have the highest family penetrance (44%), while extracellular mutations have varied degrees of penetrance. In addition, within a given family, members with the same mutation and similar degrees of CD95s defect in vitro may have very different manifestations, from full-blown disease to no symptoms at all [34]. The reasons underlying this variability are not known but may involve polymorphisms in other apoptosis-related genes. Recently, the presence of the allele HLA-B44 and certain polymorphisms in *CASP10* were shown to be protective in ALPS [35, 36].

ALPS, Type Ib

The gene mutated in ALPS Ib encodes FasL: *TNFSF6*, which is located on chromosome 1q23 and includes four exons that are translated into a 40-kD type II membrane protein. There have been three patients with ALPS Ib described to date. The first patient is an African-American man with a heterozygous, 84-bp, in-frame deletion in *TNFSF6* associated with generalized lymphadenopathy and an atypical systemic lupus erythematosus-like phenotype, including malar rash, arthritis, serositis, renal disease, and positive anti-DNA and ANA antibodies. This patient did not fulfill NIH criteria for ALPS, based on the available information [37]. In 2006, Del-Rey et al. described a woman with typical ALPS associated to a homozygous CD95L mutation causing an amino acid substitution in the intracellular portion of the molecule that abolished protein expression [38]. More recently, the NIH group reported one patient with lymphadenopathy, splenomegaly, elevated DNT cells, autoimmune thrombocytopenia and anemia, autoimmune hepatitis, and a heterozygous A530G mutation in the D95L gene that replaced Arg with Gly at position 156 in the protein's extracellular CD95-binding region. This mutation caused dominant-interfering CD95L protein that bound to the wild-type CD95L protein and prevented it from effectively inducing apoptosis [29]. In summary, disease-associated CD95L mutations include homozygous and heterozygous changes with or without CD95L protein expression.

ALPS, Type Im (mosaic)

A total of 10 patients with ALPS Im have been described so far. These patients have classical ALPS phenotype, but genetic analysis reveals heterozygous *CD95* mutations only on selected cell types, such as all DNT cells and a small percentage of CD4 and CD8 lymphocytes, monocytes, and CD34 hematopoietic precursors. The mutations are not

found on hair or mucosal epithelial cells, suggesting a somatic origin. Also, CD95-mediated lymphocyte apoptosis is normal in vitro [24]. These findings suggest that one or a few hematopoietic cell clones suffered a spontaneous D95 mutation and had a selective advantage over the normal cells, resulting in lymphoproliferation and autoimmunity. Thus, patients with sporadic forms of ALPS and normal in vitro apoptosis should have their DNT cells separated and sequenced for *CD95* mutations.

ALPS, Type II

Patients with caspase 10 gene (*CASP10*) defects are classified as type II. Caspase 10 gene is analogous to caspase 8 and linked to it on human chromosome locus 2q23. Only three patients with *CASP10* mutations have been reported to date. Their clinical phenotype is undistinguishable from other ALPS patients. Two of the patients harbored heterozygous I406L mutations and one harbored the L285F change. Like *CD95* mutations, these mutations have a dominant-negative effect on CD95 signaling when wild-type and mutant cDNA are cotransfected in lymphocytic cell lines. Despite association with disease in previous reports, the V410I mutation has now been shown to be present on 3.4% of Caucasian controls [36, 39].

Interestingly, dendritic cells from type II patients also demonstrate a defect in CD95-mediated apoptosis [39]. This raises the attractive hypothesis that the persistence of antigen-presenting cells might cause prolonged stimulation of T and B cells and contribute to the development of autoimmunity in ALPS patients. In fact, Wang et al. demonstrated in a recent paper that mice expressing a caspase inhibitor protein (p35) in dendritic cells developed more severe autoimmunity than mice expressing p35 on T or B cells [40].

ALPS, Type III

Under the designation of type III are classified patients with ALPS clinical and laboratory findings but no detectable genetic defect. Up to 23% of the patients followed at the NIH fall under this classification. Importantly, their clinical features and disease history does not differ as compared to ALPS Ia patients [31]. Typically, they have lower numbers of DNT cells and milder apoptotic defects (if present) when compared to those patients. Type III patients might harbor mutations in unknown molecules of the CD95 pathway or other apoptotic or cell cycle components.

ALPS, Type IV

We have recently described the first ALPS patient with impairment in the intrinsic pathway of apoptosis [30]. The

patient is a 49-year-old male with lifelong lymphadenopathy, splenomegaly, circulating autoantibodies, and elevated DNT cells. His clinical history was remarkable for the development of an acute leukemia at an early age and a cutaneous B-cell lymphoma at the age of 32. His lymphocytes were normally susceptible to death induced by an agonistic anti-CD95 antibody (Apo1.3) but showed a significant defect in IL-2 withdrawal-induced death, a form of cell death mediated by the mitochondrial pathway of apoptosis. The patient was found to harbor a heterozygous germline Gly13Asp activating mutation of the *NRAS* oncogene that does not impair CD95-mediated apoptosis. The increase in active, GTP-bound NRAS augmented RAF/MEK/ERK signaling, which markedly decreased the proapoptotic protein BIM and attenuated intrinsic, nonreceptor-mediated mitochondrial apoptosis. We suggest that this and future patients with defects affecting the intrinsic pathway of apoptosis be classified as ALPS type IV.

Caspase-Eight Deficiency State

In 2002, Lenardo's laboratory described a clinical syndrome caused by homozygous mutations in the gene encoding for caspase-8 (*CASP8*) affecting two siblings [41]. These patients had mild lymphadenopathy and splenomegaly, marginally elevated DNT cells, and defective D95-mediated lymphocyte apoptosis, consistent with the role of caspase-8 in the CD95 pathway; however, they were unlike other ALPS in having sinopulmonary and mucocutaneous herpes virus infections. In addition, they had low serum immunoglobulin levels, and defective activation of T cells, B cells, and natural killer cells. Given the prominent differences with classical ALPS, the disease was termed caspase-8 deficiency state (CEDS).

More recently, it was found that the impaired lymphocyte activation seen in CEDS resulted from defective activation of NF- κ B in response to stimulation through antigen receptors, TLR-4, and Fc γ RIII. This is due to the unexpected role of caspase-8 as another component of the signaling complex that includes PKC δ , Bcl-10, MALT-1, and IKK, necessary for the activation of NF- κ B in response to certain, but not all, cellular stimuli [42].

In this minireview, we have proposed that the disorders of apoptosis may provide mechanisms for the development of autoimmunity in primary immunodeficiency. An increased apoptosis and/or decreased clearance of apoptotic bodies may lead to inflammation and deficiency of Treg and, therefore, autoimmunity. In contrast, impaired apoptosis may lead to an accumulation of unique subsets of lymphocytes and failure to delete self-reactive cells and, therefore, autoimmunity, as exemplified by APLS.

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