Understanding human immunology through the study of primary immune deficiency disorders

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DISORDERS OF PROGRAMMED CELL DEATH IN LYMPHOCYTES

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INTRODUCTION

Normal immune cell homeostasis reflects a dynamic balance between cell proliferation and cell death. In a lifetime, an individual encounters numerous pathogens and each encounter perturbs immune cell homeostasis. Lymphocyte clones bearing uniquely rearranged antigen receptors have been estimated at a frequency of up to $10^{11}$ to $10^{18}$ in immunologically naïve humans. During an immune response to pathogen, antigen-specific lymphocytes rapidly expand, with an estimated 100- to 5000-fold increase in cell numbers occurring over the first week. Once infection has resolved, cell numbers rapidly decline over the first month. This contraction phase is important, because of limited immunological space.

Cells that escape death during the contraction phase become memory cells poised to fight future encounters with the same pathogen. During encounter with new antigens, a clone of specific reactivity must compete with other lymphocytes for access to antigens on antigen presenting cells, costimulatory signals, and cytokines. Thus, the presence of previously expanded but non-contracted lymphocytes could potentially hinder development of an efficient immune response to new antigens. Additionally, an excess of senescent, damaged, or autoreactive cells could predispose to autoimmunity or neoplasia. These considerations help explain why programmed cell death mechanisms exist in lymphocytes to counterbalance growth, progression through cell cycle, and division.

Programmed cell death mechanisms are highly conserved between species with homologous death genes spanning from mammals to nematodes. These genetic programs contribute to the proper development of non-immune organ systems. Studies of mice genetically deficient in certain key cell death proteins demonstrate that these mechanisms contribute to development of the mammalian nervous and reproductive systems and can be activated in non-immune cells such as hepatocytes. Investigations of humans have established that programmed cell death mechanisms are physiologically important not only during lymphocyte development, but also in proper homeostasis of mature peripheral T and B lymphocytes. This chapter will focus on our current understanding of programmed cell death mechanisms in mature lymphocytes as revealed by studies in humans having rare genetic disorders. For further details, we refer readers to several recent reviews.
FORMS OF PROGRAMMED CELL DEATH

The best studied form of programmed cell death is apoptosis, a term which literally means “falling leaves,” coined in 1972 by Kerr and colleagues. However, Virchow described this phenomenon as early as 1859. Apoptotic cells exhibit a morphology characterized by cell shrinkage and rounding, vacuolar and vesicular formation, nuclear condensation with fragmentation, membrane blebbing, and breakdown of cells into apoptotic bodies containing nuclear fragments and intact organelles (Figure 1). In vivo, apoptotic bodies are rapidly engulfed by phagocytes and do not elicit an inflammatory response. Biochemically, these changes result from a highly regulated series of molecular events, which we describe later.

Figure 1. Transmission electron micrographs showing morphologic features of dying Jurkat T cells. A normal cell is shown for comparison (A). The apoptotic cell in (B) is shrunken and displays chromatin condensation (asterisk at electron dense crescent) and many apoptotic bodies (arrows). Membrane blebbing is not seen in this image. The necrotic cell in (C) is swollen and displays numerous disintegrated organelles and disruption of its plasma membrane (arrows). The chromatin condensation (asterisk) is consistent with secondary necrosis occurring during late apoptosis. Courtesy of Dr. Lixin Zheng, NIAID, NIH.
Chapter 1

Table 1. Apoptosis

<table>
<thead>
<tr>
<th>Key concepts</th>
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<tr>
<td>• Programmed cell death is a normal physiologic process of mature peripheral lymphocytes. The best characterized form of programmed cell death is apoptosis.</td>
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<tr>
<td>• The signaling pathway for apoptosis is conserved among worms, mice, and humans. Apoptosis can proceed through an extrinsic pathway involving death receptors, or an intrinsic pathway involving mitochondria. Both pathways activate caspases in an intracellular enzymatic cascade that leads to the morphological features of cell death.</td>
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<td>• Actively cycling lymphocytes are most susceptible to death. This propriocidal mechanism is induced by excess antigen via death receptors, or when antigen becomes limiting and leads to cytokine withdrawal. These mechanisms, as well as apoptosis of dendritic cells, serve to maintain tolerance and prevent autoimmunity in vivo.</td>
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Apoptosis signaling pathways

Basic components of a conserved signaling pathway for apoptosis were first identified genetically in the nematode *Caenorhabditis elegans*. This discovery was recognized by the 2002 Nobel Prize in Physiology and Medicine, awarded to Brenner, Horvitz, and Sulston. During *C. elegans* development, 131 of the 1090 cells generated disappear in the adult animal. Several key genes – *ced-3*, *ced-4*, *ced-9*, and *egl-1* – control the death of these cells. Remarkably, these genes have mammalian homologs, which are caspases, APAF-1, BCL-2, and BH3-only proteins of the BCL-2 family, respectively. The proteins link to form a signaling pathway in which CED-3 kills, CED-4 promotes CED-3 killing, CED-9 blocks CED-4, and EGL-1 in turn blocks CED-9. These relationships between these death functions are much the same in mammals, including humans, as in nematodes.

The outcome – apoptosis – hinges critically upon its last step, activation of caspases. These key enzymes are cysteiny1 proteases that cleave after specific aspartyl residues. Although certain caspases participate in proinflammatory cytokine maturation, the rest participate in apoptosis induction. Caspases exist in the cytoplasm as inactive zymogens, so the key to their regulation is proteolytic processing and rearrangement of their conformation into a highly active form. Various stimuli can trigger the formation of signaling platforms anchored by either mitochondria-derived proteins or death receptors.
(discussed below). Oligomerization into these stoichiometric activation complexes activates initiator caspases, which in turn cleave and activate effector caspases. Effector caspases cleave substrates such as poly(ADP-ribose) polymerase (PARP), inhibitor of caspase-activated DNase (ICAD/DFF45), Rho-associated coil-coil forming kinase I (ROCK I), nuclear lamins, actin, fodrin, and keratin. These proteins function to repair DNA, maintain the integrity of plasma membrane and subcellular organelle compartments, and make up nuclear and cytoskeletal architecture. Proteolysis of substrates presumably leads to the morphologic changes seen in apoptosis, culminating in cell death.

In mammals, two principal pathways initiate apoptosis (Figure 2). The core signaling pathway elucidated in nematodes corresponds more closely to the intrinsic (mitochondrial) pathway. By contrast, the extrinsic (death receptor) pathway proceeds separately and is not found in simple invertebrates. Both intrinsic and extrinsic pathways converge at the step of caspase activation. The precisely ordered sequence of molecular events that comprise these pathways are discussed below.

Intrinsic (mitochondrial) pathway

Many physiologically important stimuli trigger the intrinsic pathway of apoptosis. These include negative selection of T cells during thymic education, growth factor or cytokine deprivation, DNA damage, and treatment with cytotoxic drugs such as chemotherapeutic agents. Proteins of the B-cell lymphoma 2 (BCL-2) family control the intrinsic pathway. The fine balance between the levels and activation status of the pro- and anti-apoptotic members of this family determines the cell’s fate. Structurally, all members of the BCL-2 family share one or more of the four known BCL-2 homology regions (BH). The pro-survival members share three or four BH regions and include BCL-2, BCL-XL, A1/BFL-1, BCL-w, BOO/DIVA/BCL-B1, and MCL-1. The pro-apoptotic members, which have two or three BH regions, structurally resemble their prosurvival relatives, and include BAX, BAK, BOK/MTD, Bcl-XS, and Bcl-G1. Finally, a subgroup of pro-apoptotic members named “BH3-only” proteins contain only one BH
region; this subgroup includes BAD, BID, BIM, BIK/NBK, BLK, HRK/DP5, Bcl-Gs, BMF, NOXA, and PUMA/BBC3.

Although certain details of how these proteins control the intrinsic apoptosis pathway remain unclear, we summarize here the most accepted current model (Figure 14.2). The BH-3 only proteins seem to act as sensors for different apoptotic stimuli. For example, BIM serves as a sensor for growth factor withdrawal, and PUMA for DNA damage. Activated BH-3 only proteins induce translocation of the pro-apoptotic protein BAX from cytosol to mitochondria, where it clusters with BAK. This leads to pore formation in the outer mitochondrial membrane, loss of inner mitochondrial transmembrane potential, and release of several apoptotic proteins such as SMAC/DIABLO, apoptosis inducing factor (AIF), and cytochrome c. Released cytochrome c oligomerizes in the cytosol with APAF-1 and pro-caspase-9 to form, in the presence of Ca\(^{2+}\) and ATP, a caspase-9-activating complex called the apoptosome. Once activated within this complex, the initiator caspase-9 cleaves and activates downstream effector caspases such as caspase-3. The resulting caspase cascade leads to cell death. It has been suggested that anti-apoptotic BCL-2 members promote survival by sequestering and inactivating BH-3 only proteins or other pro-apoptotic proteins, thereby preserving mitochondrial integrity and cell survival.

**Extrinsic (death receptor) pathway**

Apoptosis can be triggered by extracellular signals that activate cell surface death receptors of the tumor necrosis factor (TNF) receptor superfamily. Members of this superfamily have cysteine-rich extracellular domains and exist as pre-assembled trimers. The best characterized death receptors are the prototypical death receptor Fas (CD95, or Apo1) and TNFR1. Others include DR3 (Apo3), DR4, and DR5 (TRAIL-R2, or Apo2). Death receptors have cytoplasmic death domains (DD), which bind to DD-containing adaptor molecules through homotypic interactions (Figure 2). The adaptor molecule FADD is crucial for signal transduction because it also possesses a death effector domain (DED). This domain enables FADD to bind homotypically to the initiator caspases-8/10, which also contain DED. Upon receptor-ligand binding, recruitment of caspases-8/10 into
the large death-inducing signaling complex (DISC) causes their oligomerization and enzymatic activation. Clusters of DISC form higher order signaling protein oligomerization transduction structures (SPOTS), which promote further caspase activation. This leads to downstream caspase cascade for cell death.

**Figure 2. Extrinsic and intrinsic signaling pathways for apoptosis.** Two pathways exist for activating the effector caspases for lymphocyte apoptosis induction and propriocidal regulation. The extrinsic pathway activates initiator caspases-8 and -10 in death-induced signaling complexes anchored by death receptors. The intrinsic pathway activates initiator caspase-9 within the apoptosome. This structure is assembled when mitochondria are permeablized. BCL-2 family members either facilitate or antagonize mitochondrial permeability and can link the intrinsic to the extrinsic pathway. See text for more details.
Chapter 1

The extrinsic pathway can feed into the intrinsic pathway to amplify signals for death. Activated caspase-8 can cleave BID, a pro-apoptotic BH3-only BCL-2 family member analogous to the *C. elegans egl-1* gene. Truncated BID improves pro-apoptotic multi-domain BAX and BAK binding to mitochondrial membranes, which increases mitochondrial permeability. Cells that depend upon this mitochondrial amplification for death-receptor mediated death include peripheral blood lymphocytes. By contrast, other cell types that exhibit more efficient DISC formation for caspase-8 and downstream caspase-3 activation die independently of mitochondrial involvement.

Engagement of death receptors can trigger other cellular responses besides death. For example, Fas stimulation also activates the transcription factor NF-κB and mitogen-activated protein kinases (MAPK) p38 and ERK1/2. Activation of these signaling pathways can counterbalance signals for death. However, Fas mutations generally impair death signals more so than growth-promoting signals.

*Lymphocyte death during an immune response*

Mature peripheral lymphocytes vary in their susceptibility to apoptosis. Resting cells and cells undergoing initial activation are typically refractory to death. This property allows an effective immune response to develop against pathogens. Once cells proliferate and enter late G1/S phase of the cell cycle, they become sensitive to death. The acquisition of death sensitivity occurs during the height of an ongoing immune response and at its conclusion. Thus, lymphocytes possess a built-in rheostat that controls cell death depending upon how rapidly they progress through cell cycle.

The sensitivity of lymphocytes to antigen-driven cell death during an immune response, termed propriocidal regulation, is a major negative feedback mechanism controlling the intensity of immune responses. Two events contribute to antigen receptor restimulation-induced propriocidal death (Figure 3). First, high levels of antigen stimulate the production of high levels of interleukin (IL)-2, which drives lymphocytes into cell cycle. Cells respond by undergoing molecular changes rendering them susceptible to the extrinsic pathway or “active” apoptosis. This process is independent of new protein or mRNA synthesis, but may involve increased Fas expression and
downregulation of anti-apoptotic molecules such as c-FLIP. As a consequence, T and B lymphocytes are triggered to die when their Fas receptors interact with soluble or membrane bound FasL. CD8 cells also die when triggered through their TNF receptors. In addition, the antigen receptor appears to directly connect to the pro-apoptotic molecule BIM. This mechanism helps limit the magnitude of the immune response, and thereby protects the host in the presence of continuous or repeated antigenic stimulation.

The second mechanism contributing to death of lymphocytes occurs when an immune response wanes (Figure 3). A falling level of antigen markedly decreases the amount of IL-2 produced. This in turn decreases CD25 – a component of the high affinity IL-2 receptor complex whose expression is IL-2-dependent – and renders the cells increasingly non-responsive to IL-2. Cytokine withdrawal drives the intrinsic pathway of apoptosis in a process that requires new protein and mRNA synthesis. Death can be blocked by addition of common g chain receptor cytokines besides IL-2, such as IL-4, IL-7, and IL-15, or by overexpressing anti-apoptotic BCL-2/BCL-XL proteins that restrain apoptosis at the mitochondria. This mechanism facilitates contraction of the immune response when activated lymphocytes have eliminated pathogen and become superfluous.

In summary, actively cycling lymphocytes die through mechanisms involving the antigen receptor-induced death mediated partly by death receptors Fas and TNF, as well as cytokine withdrawal. These complementary mechanisms limit the magnitude and duration of an immune response by eliminating activated cells.
Figure 3. Dynamics of lymphocyte apoptosis. Propriocidal regulation of actively cycling lymphocytes involves apoptosis induced through 1) death receptors at the height of the immune response, and 2) upon cytokine withdrawal at the end of the response.

In vivo importance of apoptosis

Studies in experimental animals and in humans established that apoptosis is a normal physiological process that maintains immunologic tolerance and prevents development of autoimmunity. Lpr (lymphoproliferation) is a naturally arising mouse strain with a mutation in Fas resulting in its deficiency. Homozygous mutant mice develop splenomegaly and lymphadenopathy. Depending upon genetic background, they also develop hypergammaglobulinemia, autoantibodies, glomerulonephritis, polyarteritis, sialoadenitis, arthritis, or primary biliary cirrhosis. Similar disease features are seen in another naturally arising mouse strain called gld (generalized lymphoproliferative disease), which has a homozygous Fas ligand (FasL) mutation. Recently, mice were generated in which the Fas death receptor pathway is selectively blocked in different immune cells. Surprisingly, disease was most severe in the Fas-blocked dendritic cells as compared to lymphocytes. Dendritic cell numbers and antigen presentation cell (APC) function were increased. These results indicate that death of dendritic cells, acting in
concert with propriocidal death of lymphocytes, serves to enforce tolerance and prevent autoimmunity in vivo.

THE AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

Clinical features

In 1992, Sneller and colleagues described a childhood syndrome of immune cell dysregulation including autoimmunity, hypergammaglobulinemia, lymphadenopathy, and lymphocytosis, including expansion of an unusual T cell population bearing rearranged TCR-α/β but lacking CD4 or CD8 co-receptor expression (double-negative T cells, DNT). This constellation of findings resembled key phenotypic features of lpr and gld mouse strains, natural models of autoimmune disease later found to harbor Fas and Fas ligand mutations, respectively. Like lpr mice, the patients with immune cell dysregulation had FAS mutations leading to the defective lymphocyte apoptosis that was critical for disease pathogenesis. The human disease was therefore termed autoimmune lymphoproliferative syndrome (ALPS) (Table 2), and is likely the same clinical entity characterized in descriptive accounts by Canale and Smith and others in the earlier literature.

Table 2. Clinical relevance of ALPS

- Autoimmune lymphoproliferative syndrome (ALPS) is a genetic disorder that impairs lymphocyte apoptosis.
- Mutations in FAS underlie most cases of ALPS. A minority of ALPS is caused by mutations in FASL (Fas ligand), CASP10 (caspase 10), or somatic FAS mutations affecting DNT cells. These mutations all impair the Fas-mediated extrinsic pathway of apoptosis.
- ALPS predisposes to autoimmunity and lymphomas. The most typical autoimmune findings are autoantibodies, Coombs positive hemolytic anemia, or chronic idiopathic thrombocytopenic purpura. Hodgkin and non-Hodgkin lymphomas both occur.
- Caspase-8 deficiency state (CEDS) is characterized by combined lymphocyte immunodeficiency with susceptibility to infection, superimposed upon ALPS-like features of lymphoproliferation and apoptotic defects. These findings stem from the participation of caspase-8 in two different signaling complexes – one for death induction, and another for NF-kB activation.
ALPS is a rare condition with variable disease penetrance, affecting a reported 300 persons worldwide. Diagnostic criteria, discussed below, reflect deranged lymphocyte homeostasis (Table 3; Table 4). Patients must present with chronic non-malignant lymphocyte accumulation, including lymphadenopathy and/or splenomegaly, elevated DNT numbers in peripheral blood, and defective in vitro lymphocyte apoptosis. Autoimmunity is often seen, or more rarely lymphoma, but these are not required for diagnosis.

Table 3. Diagnostic criteria for ALPS

<table>
<thead>
<tr>
<th>Required criteria</th>
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<tr>
<td>-Chronic non-malignant lymphadenopathy and/or splenomegaly</td>
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<tr>
<td>-Increased peripheral CD4&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;-&lt;/sup&gt; TCRα/β (DNT) cells</td>
<td></td>
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<tr>
<td>-Lymphocyte apoptosis defect</td>
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<tr>
<th>Supporting criteria</th>
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<tr>
<td>-Family history of ALPS</td>
<td></td>
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<tr>
<td>-Characteristic histopathology</td>
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<tr>
<td>-Autoimmune manifestations</td>
<td></td>
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<tr>
<td>-Mutations in FAS, FASLG, CASP10</td>
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Signs and symptoms of ALPS usually emerge in infancy or early childhood (median age of 24 months), when patients undergo medical investigation for unexplained splenomegaly, lymphadenopathy, or autoimmune destruction of blood cells. Typically there is painless enlargement of peripheral lymphoid organs, with no fever or weight loss unless complicated by lymphoma. The thymus or liver can also be enlarged. Although lymphoid hyperplasia can fluctuate, in general it gradually improves with age, even without treatment. Up to 80% of ALPS patients have circulating autoantibodies, although only half have actual autoimmune disease. Coombs positive hemolytic anemia and chronic immune thrombocytopenic purpura are the most common autoimmune diseases. These manifestations tend to be severe, but often follow a variable course. When seen at initial presentation, ALPS can be mistaken for Evans syndrome. Neutropenia can occur, caused either by hypersplenism or of autoimmune origin, but is usually mild. The high incidence of anticardiolipin or anti-neutrophil antibodies has no correlation with clinical manifestations of thrombosis or neutropenia. Rare autoimmune manifestations reported
include antinuclear antibodies, rheumatoid factor, glomerulonephritis, optic neuritis or uveitis, Guillain-Barré, primary biliary cirrhosis, anti-factor VIII antibodies with coagulopathy, autoimmune hepatitis, vasculitis, and linear IgA dermopathy.

Table 4. ALPS: Clinical pearls

- Classification is based upon findings that may be present or have resolved.
- Despite its moniker, only up to 80% of patients with ALPS have evidence of autoimmunity. In other words, lack of autoimmunity does not preclude ALPS.
- The variable penetrance means that not all individuals with mutations manifest disease.
- TCRγ/δ cells lacking CD4 and CD8 co-receptors may falsely elevate the double negative T cell (DNT) count and therefore should be excluded. Expression of B220 on DNT cells is a finding specific to ALPS.
- Elevated B12 levels can be used as a simple screen. Other helpful laboratory findings are hypergammaglobulinemia, direct Coombs test, or anti-cardiolipin antibodies.
- Patients without known mutations should be directly analyzed for somatic mutations in sorted DNT cells. Apoptosis cannot be readily assessed by the usual methods, because these cells cannot be maintained in culture.

DNT expansion is peculiar to and required for diagnosis of ALPS. Normally, DNT comprise less than 1% of lymphocytes in peripheral blood or lymphoid tissue, but can reach up to 40% in ALPS patients. These cells are distinct from the immature DNT cells developing in the thymus that have not yet rearranged the genes for or expressed antigen receptors. They are thought to represent aging mature T cells that have lost CD4 or CD8 co-receptor expression, but the details of their origin are obscure. DNT express the CD45R isoform B220, a marker normally found on B cells, as well as CD27, CD57, and human leukocyte antigen (HLA)-DR. In ALPS patients the expanded DNT produce high levels of IL-10. As elevated IL-4 and IL-5 are also seen, the resulting T helper type 2 (Th2) cytokine profile probably contributes to the observed polyclonal hyperglobulinemia and autoantibodies. The demonstration that some ALPS patients are mosaics – with Fas mutations in DNT but not all T cells – implicates DNT in disease pathogenesis. However, understanding their exact function has been hampered by an inability to keep them alive in culture, as they do not respond well to most activating and proliferative stimuli.
Lymphoproliferation in ALPS is not isolated to DNT. Patients have increased numbers of total T cells, with contributions from CD8 cells expressing CD57 as well as TCRγ/δ+ cells that lack CD4 and CD8 expression. Both total B cells and CD5+ B cells are increased. By contrast, CD4+CD25+ cells are low, resulting in no overall change in CD4 cell numbers. NK cell numbers are also unchanged. Lymph node biopsy reveals a characteristic histopathology showing follicular hyperplasia with polyclonal plasmacytosis, and paracortical expansion with infiltrating DNT. Besides lymphocyte immunophenotyping, high levels of circulating cobalamin (vitamin B12) can be used as a simple initial screening test for ALPS, but by itself cannot differentiate from certain other hematologic disorders including lymphoproliferative and myeloproliferative disorders (V.K. Rao, J.K. Dale, and S.E. Straus, personal communication).

A definitive diagnosis of ALPS requires the evaluation of lymphocyte apoptosis in vitro, a test performed only in specialized research laboratories such as the National Institutes of Health in Bethesda, MD, USA. A key point for the appropriate interpretation of this test is that cells must be proliferating well in order to become susceptible to death-inducing stimuli. Thus, poor proliferation can be falsely interpreted as an apparent apoptosis resistance. We typically activate peripheral mononuclear blood leukocytes with phytohemaglutinin (PHA) and then drive T cells into cell cycle by culturing for several more days with IL-2. Most laboratories use Fas agonistic antibodies or TCR restimulation to induce apoptosis. After induction of apoptosis, a flow cytometer is used to count propidium iodide-excluding cells (“live” cells) over constant time. This number is compared to that for untreated cells to calculate a percent cell loss at any given dose of stimulus. A decrease in percent cell loss at half of normal controls is usually considered evidence of an apoptosis defect.

Although initially non-malignant, the lymphoproliferation in ALPS predisposes to lymphoid malignancy, which develops in 10% of ALPS patients. In rare instances, ALPS patients are diagnosed when they initially present with lymphoma. Compared to the general population, ALPS patients with FAS mutations (type 1A) have a 51-fold and 14-fold elevated incidence of Hodgkin and non-Hodgkin lymphoma, respectively. Median age of diagnosis was 11 years for Hodgkin lymphoma and 21 for non-Hodgkin lymphoma; however, lymphomas were identified anywhere between 2 to 50 years of age.
Lymphomas are of either B or T cell origin and of diverse histological type, thus betraying a general anti-neoplastic role for propriocidal death of lymphocytes. The lymphomas display neither loss of heterozygosity for the Fas mutation nor increased apoptosis resistance. Most patients who develop malignancy have mutations in the DD region, with severe impairment in Fas-mediated lymphocyte apoptosis but continued Fas-mediated activation of NF-κB and MAPK for growth promotion.

**Molecular etiology and classification**

A proposed ALPS classification based on underlying genetic defects is presented in Table 5 and the corresponding molecular mechanisms are discussed below.  

<table>
<thead>
<tr>
<th>Classification</th>
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<td>Type Ia</td>
<td>FAS (TNFRSF6)</td>
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<tr>
<td>Type Ib</td>
<td>FASLG (TNFSF6)</td>
</tr>
<tr>
<td>Type Im</td>
<td>somatic FAS mutations</td>
</tr>
<tr>
<td>Type II</td>
<td>CASP10</td>
</tr>
<tr>
<td>Type III</td>
<td>Unknown</td>
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Most ALPS patients bear heterozygous mutations in the *FAS (TNFRSF6)* gene located on chromosome 10q24.1. Mutations can be found throughout the gene, either in coding regions or in splice sites, with the majority (~2/3) affecting the intracellular death domain (DD) encoded by exon 9. (A description of all mutations can be found in the ALPS database [ALPSbase: http://research.nhgri.nih.gov/ALPS/].) Autosomal dominant disease transmission occurs because most mutations exert a dominant interfering effect. This effect is explained by understanding that pre-association of three normal Fas molecules in a trimer is required for receptor death signaling function. If 50% of Fas proteins are abnormal, as is the case for heterozygous mutations, seven out of eight Fas trimers would contain at least one mutant protein, rendering that trimer ineffective. Severity is greatest for DD mutations, which disrupt the homotypic interactions required for FADD and initiator caspase recruitment into the death-inducing signaling complex (DISC). Those that do not affect DISC formation can impair downstream higher order
signaling protein oligomerization transduction structures (SPOTS) formation, which is necessary for efficient caspase activation. A few ALPS patients have compound heterozygous loss-of-function mutations that cause haploinsufficiency. Individuals with complete loss of function due to homozygous mutations have generally more severe symptoms. ALPS patients with FAS mutations are classified as type Ia. In some ALPS patients, FAS somatic mutations have been found in purified DNT cells and a fraction of peripheral lymphocytes, monocytes and hematopoietic precursors. Notably, their (non-DNT) T cells lacked FAS mutations and apoptosis defects when expanded in vitro. These patients are provisionally classified as type I_m (for “mosaic”).

A minority of ALPS patients harbor mutations in other components of the Fas pathway. A heterozygous mutation in Fas ligand (FASLG, TNFSF6) was originally reported for a patient with systemic lupus erythematosus (SLE), who had lymphadenopathy, splenomegaly, and defective lymphocyte apoptosis after TCR restimulation, but no apparent DNT expansion. However, ALPS patients were also discovered who possessed either heterozygous (JK Dale and SE Straus, personal communication) or a homozygous FASLG mutations\(^\text{19}\), and these are classified as type 1b. Although caspase-10 functional polymorphisms may influence disease, at least two heterozygous caspase-10 mutations in three ALPS patients have been identified that cause defective apoptosis in lymphocytes and dendritic cells.\(^\text{20}\) These patients have been classified as type II.

A subgroup of ALPS patients fulfills diagnostic criteria including increased DNT but lacks detectable Fas-mediated apoptosis defect or known mutations in the Fas pathway. These have been termed type III. We have recently identified one such patient who demonstrated defective lymphocyte apoptosis upon cytokine withdrawal, a stimulus that triggers the intrinsic pathway (JB Oliveira, TA Fleisher, and MJ Lenardo, personal communication). The molecular defect underlying disease in this patient is currently under intensive investigation.

The relationship between genotype and phenotype is complex. Gene mutations are required, but are not sufficient for disease. In large kindreds, family members with the same mutation and degree of defective in vitro Fas-mediated apoptosis had very different clinical manifestations. In-depth analysis revealed that penetrance was greatest with
intracellular DD mutations and least with extracellular mutations. This data indicates that other factors, genetic and/or environmental, influence the clinical phenotype.

**ALPS-like disease**

Some patients have features overlapping with but not fulfilling diagnostic criteria for ALPS. For instance, the ALPS-variant autoimmune lymphoproliferative disease (ALD) has lymphoproliferation, autoimmune disease, susceptibility to cancer, and defective lymphocyte apoptosis, but lacks DNT expansion. The responsible molecular defects are unknown. In another cohort patients display clinical features of ALPS but lack apparent apoptosis defects. Apoptotic defects are usually assessed through the Fas death receptor, which may not reveal defects that would be seen if induced by other stimuli. Apoptotic defects may also not be readily apparent depending upon the cells used for analyses, for example in somatic mosaicism. Given recent exciting demonstrations using transgenic mouse models that dendritic cell apoptosis defects can contribute to disease pathogenesis, we speculate that other diseases resembling ALPS, such as Rosai-Dorfman disease, may involve mutations within antigen presenting cells. In sum, a proportion of ALPS-like disease likely represents variants of ALPS. However, other forms of ALPS-like disease probably reflect abnormalities in non-apoptotic pathways that also regulate lymphocyte growth and activation.

There is a growing realization that certain molecules involved in apoptosis also function integrally for lymphocyte growth and activation. One such molecule is caspase-8. Its deficiency, found in two siblings with homozygous mutations, leads to an immune regulation syndrome we term caspase-8 deficiency state (CEDS). Consistent with the known role of caspase-8 as an initiator caspase in the DISC, these patients had mild lymphadenopathy and splenomegaly, as well as lymphocyte apoptotic defects. However, they had inconsistent marginally elevated DNT, raising doubt as to whether they fulfilled ALPS criteria. More importantly, unlike classical ALPS patients, the CEDS patients had a prominent combined immunodeficiency with recurrent sinopulmonary infections and mucocutaneous herpesvirus infections. They had low serum immunoglobulin levels and poor humoral responses to polysaccharide antigens, as well as impaired activation of T
cells, B cells, and natural killer (NK) cells. Thus, they resembled patients with common variable immunodeficiency (CVID). We recently found that the impaired lymphocyte activation resulted from a defect in the kinetics of activation of the critical transcription factor NF-κB in response to stimulation through antigen receptors, TLR-4, and FcγRIII. This is because caspase-8 participates in a signaling complex that includes other proteins such as Bel-10, MALT-1, and IKK in an NF-κB activating signalosome. This complex differs from the DISC and is not affected in classical ALPS patients. It will be important to identify other patients with caspase-8 deficiency to allow us to define further the spectrum of this disease given the potential for diagnostic confusion.

**Apoptosis in other immunodeficiencies**

Although resting lymphocytes are relatively resistant to death, they appear to require antigen receptor expression with continuous low or intermittent stimulation, in addition to homeostatic cytokines such as IL-7 and IL-15 for survival. Mice rendered genetically deficient in anti-apoptotic BCL-2 family members (such as BCL-2, BCL-X, and MCL-1), show loss of mature or developing lymphocytes. These results suggest that inappropriately activated intrinsic pathways of apoptosis may contribute to immunodeficiency. Several studies demonstrated increased spontaneous apoptosis of lymphocytes from patients with CVID, ataxia-telangiectasia, adenosine deaminase-severe combined immunodeficiency (ADA-SCID), Omenn’s syndrome, cartilage-hair hypoplasia, and DiGeorge syndrome. However, without defining biochemically how specific gene mutations affect apoptosis, it is difficult to know whether these associations are simply correlative or indeed causative.

**Therapies for ALPS**

Although symptoms can remit with age, some ALPS patients require continued treatment to control autoimmune disease (Table 6). Treatment for autoimmune cytopenias is similar to that used in patients without ALPS. A high-dose pulse of corticosteroid (5-30 mg/kg methylprednisolone i.v.) is useful in bringing autoimmune cytopenias rapidly under control. This is followed by a low-dose course (1-2 mg/kg prednisone orally) that
can be tapered and eventually discontinued after several weeks to months. Adjuncts to corticosteroids include IVIG for autoimmune thrombocytopenia or hemolytic anemia, and granulocyte-colony stimulating factor (G-CSF, 1-2 mg/kg from three times a week to once daily) for autoimmune neutropenia. In some patients, autoimmune disease promptly recurs after discontinuing corticosteroids; these patients may need to be maintained on a minimal dose every other day. Alternatively, such patients may benefit by switching to the immunosuppressant mycophenolate mofetil (~600 mg/M$^2$/dose orally, twice daily) for long-term maintenance therapy. The long-term corticosteroid- and splenectomy- sparing effects of this agent are particularly advantageous in children. For patients who fail these approaches, cytotoxic agents can be tried. Success has been reported using azathioprine, vincristine, or rituxan (anti-CD20 monoclonal antibody, 375 mg/M$^2$/week i.v. X 4), although no controlled prospective trials exist to support the widespread use of these drugs in ALPS patients. Allogeneic bone marrow transplantation has been curative when undertaken in the rare instances of severely and intractably affected patients with homozygous FAS mutations and complete absence of Fas. However, given the associated high risks of complications and death, use of matched unrelated donor allogeneic bone marrow – which is likely to be required to avoid repopulating with lymphocytes from family members having the same mutation – should be considered a therapy of last resort.

Although lymphadenopathy and splenomegaly may be unsightly, these disease manifestations are usually not treated unless medically indicated. Hypersplenism can contribute to low blood cell counts when splenic pooling exacerbates autoimmune-mediated destruction. Notably, none of the current agents used to treat ALPS improves lymph node or spleen size. Splenectomy may be required if immunosuppressant agents fail to improve cytopenias. A disproportionately increased risk for post-splenectomy pneumococcal sepsis and death in ALPS patients means that splenectomy should be undertaken only after extensive discussions with the patient and family. Patients should be immunized with vaccines against Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis before splenectomy. Following splenectomy, patients should be re-immunized with conjugate plus polysaccharide pneumococcal vaccines when titers wane. Lifelong antibiotic prophylaxis with penicillin or fluoroquinolones is essential.
Moreover, splenectomized patients should be instructed to seek immediate medical attention to rule out bacteremia during febrile illnesses.

**Table 6. Therapeutic principles**

- Disease can improve with age.
- Corticosteroids are useful for rapidly bringing autoimmune disease under control.
- If unable to discontinue medications, low-dose corticosteroids given every other day, or mycophenolate mofetil, may be useful in preventing recurrence of autoimmune disease.
- Splenectomy may be considered only when hypersplenism contributes to severe refractory cytopenias.
- Risk of post-splenectomy sepsis necessitates lifelong antibiotic prophylaxis.
- Cytotoxic agents or matched unrelated donor allogeneic bone marrow transplantation can be considered for worst case scenarios.
- Patients should undergo periodic monitoring for development of lymphoma.
- Lymphomas respond to conventional therapies.

Given that treatment of ALPS patients aims primarily at controlling autoimmune manifestations, drugs that control lymphoproliferation or prevent lymphomagenesis are needed. Several case reports suggested that sulphadoxine-pyrimethamine (Fansidar) treatment might have utility in patients with ALPS or ALPS-like disease. Regression of lymphadenopathy and/or splenomegaly, as well as improvement in blood cell counts, were observed in six of seven treated patients. Two of the treated patients sustained remission after discontinuing the drug. However, more recent studies in ALPS patients and the lpr mouse model failed to demonstrate any significant benefit of this agent or pyrimethamine alone on lymph node or spleen size (VK Rao, KC Dowdell, and SE Straus, personal communication). Therefore, at present this regimen cannot be considered recommended therapy.

Finally, the predisposition to lymphoma presents a unique clinical challenge: how to distinguish a newly developing or relapsed lymphoma from benign lymphadenopathy. ALPS patients require careful periodic examinations and surveillance by serial computed tomography (CT) scans. Suspiciously enlarging lymph nodes may necessitate biopsy to assess for clonality and chromosomal abnormalities. Positron emission tomography (PET) scans, which detect areas of high cellular glucose uptake, are useful for identifying
and following suspicious lesions. Fortunately, lymphomas in ALPS patients respond to conventional therapies and do not have a worse outcome.

CONCLUSION

Programmed cell death is an essential regulatory mechanism to establish equipoise with growth, differentiation, and proliferation of lymphocytes. Studies in humans with the rare genetic disorder autoimmune lymphoproliferative syndrome (ALPS) have demonstrated that apoptosis is physiologically important for maintaining lymphocyte homeostasis, preventing autoimmunity, and suppressing lymphomagenesis. Studies in ALPS-like disorders such as caspase-8 deficiency state (CEDS) have revealed that molecules responsible for death can also participate in other intracellular signaling pathways for normal lymphocyte function. Defining the genetic abnormalities responsible for ALPS and related disorders will continue to provide insights into the mechanisms that regulate immune cell homeostasis in vivo.

References
Chapter 1


