



Monogenic defects in lymphocyte apoptosis

Thomas A. Fleisher and Joao Bosco Oliveira

Purpose of review

The recognition that apoptosis – programmed cell death – is an important mechanism in immune homeostasis has led to the identification of human disorders associated with defects in the critical control mechanism.

Recent findings

Patients have been identified with defects affecting the extrinsic apoptotic pathway mediated by the protein receptor FAS which results in the autoimmune lymphoproliferative syndrome and more recently in defects affecting the intrinsic apoptotic pathway mediated by RAS proteins resulting in the RAS-associated autoimmune leukoproliferative disorder. This review summarizes the immunopathogenesis, clinical features and diagnostic approaches to these human disorders.

Summary

Apoptotic pathways are critical in the maintenance of leukocyte homeostasis, and genetic defects impacting these can result in clinical disease manifested as expansion of selected leukocyte populations, autoimmunity, increased risk for malignancy and in some situations defects in host defense.

Keywords

ALPS, apoptosis, FAS, RALD, RAS

The recognition that apoptosis – the process of programmed cell death – plays an important role in immune homeostasis has been complimented by the identification of human defects of apoptosis affecting the immune system [1]. These disorders typically present with select immune cell expansion, autoimmunity and increased risk for malignancy, but also may be associated with increased risk of infection [1]. The first description of this group of disorders was the autoimmune lymphoproliferative syndrome (ALPS), caused by genetic defects affecting the extrinsic pathway of lymphocyte apoptosis [2,3]. This apoptotic pathway is controlled by several receptor-ligand pairs of the tumor necrosis factor (TNF) receptor superfamily, and in the immune system provides one mechanism for controlling the extent of antigen-specific lymphocyte responses [4]. Among these receptors, one that is critically important for lymphocyte homeostasis is the FAS receptor (CD95, Apo-1) and its specific ligand (FASL) [4]. FAS is up-regulated upon T-cell activation and appears to be a critical signaling molecule for apoptosis associated with T-cell receptor (TCR) restimulation; it is also involved in the elimination of self-reactive lymphoid cells and provides one pathway for inducing immune-based cytotoxicity directed against specific target cells [4,5]. Since the initial description and investigation

of FAS defects associated with ALPS, additional work has uncovered other defects within the FAS–FASL pathway that result in human disease [1]. More recently, a new disorder that has some overlapping features with ALPS as well as unique clinical features has been identified that is associated with defects in the RAS (rat sarcoma family) proteins (KRAS and NRAS), ultimately affecting the intrinsic pathway of apoptosis [6⁷,7,8].

A link between lymphocyte cell death and autoimmunity was first revealed by studies in the *lpr* and *gld* murine models of systemic lupus erythematosus demonstrating that the autoimmune disease was associated with defective lymphocyte apoptosis [9]. This was followed by work defining that mutations in the genes encoding either Fas (*lpr*) or FasL (*gld*) caused the autoimmunity and lymphoproliferation observed in these murine models [10,11]. Disease in these mice includes the development of massive lymphadenopathy, antinuclear

NIH Clinical Center, National Institutes of Health, Bethesda, Maryland, USA

Correspondence to Thomas A. Fleisher, MD, Department of Laboratory Medicine, NIH Clinical Center, NIH, Building 10, Room 2C410, Bethesda, MD 20892-1508, USA. E-mail: tfleishe@mail.nih.gov

Curr Opin Allergy Clin Immunol 2012, 12:000–000

DOI:10.1097/ACI.0b013e3283588da0

KEY POINTS

- Monogenic defects in apoptosis typically present with lymphoaccumulation and specific autoimmunity.
- ALPS due to *FAS* mutations is associated with elevations in circulating levels of interleukin (IL) -10, sFASL and vitamin B12.
- Autoimmune lymphoproliferative syndrome due to germline *FAS* mutations is associated with a significant increase in the risk for developing lymphoma.
- RALD is associated with a monocytosis that is uniquely seen in ALPS and is not observed in ALPS.

antibodies, circulating immune complexes with resultant glomerulonephritis and a striking increase in circulating CD3⁺CD4⁺CD8⁺ T cells that express the alpha-beta T-cell antigen receptor (α/β -TCR). These findings are associated with homozygous mutations in one of these two genes, but the extent of disease is strain-dependent [10,11]. This work established an important role of the FAS-dependent extrinsic apoptotic pathway in controlling auto-reactivity and paved the way for the discovery of the genetic basis of disease in humans presenting with a number of features in common with the murine disorder.

AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME

In 1967, Canale and Smith [12] described a group of patients who presented in early childhood with generalized lymphadenopathy and hepatosplenomegaly associated with autoimmune anemia, thrombocytopenia and increased gammaglobulins. In 1992, two additional patients were reported with similar clinical features and a marked increase in circulating α/β -TCR⁺ T cells that did not express CD4 or CD8 [termed double-negative (DNT) cells], that normally constitute less than 1.5% of peripheral blood lymphocytes [13]. The investigators then noted the similarity between the findings in these patients and the *lpr/gld* murine models. The hallmark features were presentation in childhood with nonmalignant lymphoaccumulation and autoimmunity, together with an expansion of $\alpha\beta$ -DNT cells, and ultimately this disorder was defined as the ALPS [13].

Genetics and pathophysiology

Following the discovery of the genetic defect in the *lpr* mouse model, the corresponding gene in

humans, *FAS* (*TNFRSF6*, *APO1*), was evaluated and damaging heterozygous mutations were found in many patients diagnosed with ALPS [2,3,14]. This gene consists of nine exons, the first five of which code for three cysteine-rich extracellular domains characteristic of the TNF-receptor family [15]. The sixth exon codes for a transmembrane domain, and the last three (exons 7–9) codes for the intracellular domain. The intracellular domain contains the critical death domain coded for by exon 9, the site of the protein that is directly involved in the apoptotic signaling pathway. The majority of ALPS patients have a heterozygous mutation in *FAS* with an autosomal dominant inheritance pattern due to a DNT effect, contrasting with the *lpr* model, which is an autosomal recessive disorder [2,16[•],17[•]]. This effect is explained by work demonstrating that the functional FAS receptor is a preassembled homotrimer [18]. If 50% of FAS protein is abnormal, as is the case for heterozygous mutations, the majority (i.e. 7 of 8) of FAS homotrimers would contain at least one mutant protein, rendering that complex ineffective. A handful of ALPS cases caused by homozygous or compound heterozygous germline mutations in *FAS* have been reported and, as might be expected, manifest more aggressive disease presenting during the neonatal period [19,20].

A number of patients with typical clinical features of ALPS were, however, found to have a normal *FAS* gene leading to studies directed at the genes encoding FASL and selected intracellular proteins involved in this apoptotic pathway. This work identified a minority of patients with mutations in the *FASL* gene (*TNFSF6*) and the cystinyl-aspartate-requiring proteinase (caspase) 10 gene (*CASP10*) [21–24]. More recently, somatic mutations in *FAS* have been identified and are now known to represent the second most common genetic cause of ALPS [25,26[•]]. Additionally, specific biomarkers including elevation in FASL, IL-10 and vitamin B12 have been identified as associated with ALPS due to either germline or somatic mutations in *FAS* [27,28^{••}]. These findings form the basis of the current diagnostic criteria (given below) and classification scheme for ALPS (Table 1) [29].

Diagnostic criteria for ALPS (A definitive diagnosis is based on the presence of both required criteria and one primary accessory criterion. A probable diagnosis is based on the presence of both required criteria and one secondary accessory criterion.):

- (1) Required
 - (a) Chronic (>6 months), nonmalignant, non-infectious lymphadenopathy or splenomegaly or both

Table 1. Genetic classification of ALPS

ALPS-FAS	Germline mutation in the gene encoding FAS (<i>TNFRSF6</i>)
ALPS-sFAS	Somatic mutation in the gene encoding FAS (<i>TNFRSF6</i>)
ALPS-FASLG	Mutation in the gene encoding FASL (<i>TNFSF6</i>)
ALPS-CASP10	Mutation in the gene encoding caspase 10 (<i>CASP10</i>)
ALPS-U	No known mutation

ALPS, autoimmune lymphoproliferative syndrome.

- (b) Elevated $CD3^{+}TCR\alpha\beta^{+}CD4^{-}CD8^{-}$ DNT cells ($\geq 1.5\%$ of total lymphocytes or 2.5% of $CD3^{+}$ lymphocytes) in the setting of normal or elevated lymphocyte counts;
- (2) Primary accessory
 - (a) Defective lymphocyte apoptosis (in two separate assays)
 - (b) Somatic or germline pathogenic mutation in *FAS*, *FASLG* or *CASP10*; and
- (3) Secondary accessory
 - (a) Elevated plasma sFASL levels (>200 pg/ml) or elevated plasma IL-10 levels (>20 pg/ml) or elevated serum or plasma vitamin B12 levels (>1500 ng/l) or elevated plasma IL-18 levels above 500 pg/ml
 - (b) Typical immunohistological findings as reviewed by an experienced hematopathologist
 - (c) Autoimmune cytopenias (hemolytic anemia, thrombocytopenia or neutropenia) and elevated immunoglobulin G levels (polyclonal hypergammaglobulinemia)
 - (d) Family history of a nonmalignant/non-infectious lymphoproliferation with or without autoimmunity.

As the number of patients diagnosed with ALPS has increased it has become very clear that family members within individual pedigrees with identical heterozygous mutations may have few, if any, features of ALPS [30]. A review of the clinical and genetic information revealed that the site of the *FAS* mutation impacted the disease penetrance and severity with mutations affecting the intracellular domains (exons 7–9) associated with a higher incidence of ALPS-associated features and greater morbidity compared with those patients having extracellular *FAS* mutations [31,32]. Consistent with these differences is the finding that in many cases of ALPS associated with extracellular mutations, the mechanism involved is one of haploinsufficiency, which may explain the decreased severity and

penetrance observed in these patients [17[■],33]. Interestingly, Rieux-Laucat *et al.* have recently demonstrated that the development of autoimmunity in patients with less severe germline *FAS* mutations may be induced by the appearance of somatic mutations affecting the healthy *FAS* allele [34].

Clinical and laboratory findings

The typical clinical course in ALPS begins with nonmalignant, noninfectious-based peripheral lymphadenopathy often associated with splenomegaly that develops within the first 5 years of life [16[■],35,36]. However, many patients have been diagnosed later including during adulthood. The lymphoaccumulation usually fluctuates over time and tends to improve after adolescence. Autoimmunity is primarily directed at blood cells, presenting as autoimmune hemolytic anemia, thrombocytopenia and/or neutropenia that is often exacerbated by hypersplenism. These findings are associated with autoantibodies to the blood cells commonly with a positive direct Coombs' test and antiplatelet or antineutrophil autoantibody tests [37]. The immune cytopenias can be significant and resistant to first-line treatment, requiring drugs such as mophetil mycophenolate or rapamycin for adequate control [38]. There have been infrequent reports of other autoimmune manifestations including uveitis, autoimmune hepatitis, Guillain-Barré and systemic lupus erythematosus.

Another feature of ALPS is a dramatically increased incidence of lymphoma in patients with germline *FAS* mutations manifest as an increased relative risk of 51-fold for Hodgkin disease and 14-fold for non-Hodgkin's lymphoma [39]. These lymphomas are typically of B-cell origin and respond to conventional treatment regimens. There is not sufficient data at this time as to whether or not somatic *FAS* mutations are associated with an increased risk for lymphoma.

The most remarkable laboratory finding is the presence of an expanded population of $\alpha\beta$ -DNT cells as measured by flow cytometry, and this finding represents a required diagnostic criterion (listed above under the 'Genetics and pathophysiology' subsection) [29]. Other common immunophenotypic findings include expansion of $CD3^{+}HLA-DR^{+}$ cells, low memory B cells ($CD20^{+}CD27^{+}$) and increased $CD20^{+}CD5^{+}$ B cells, in the presence of normal or elevated lymphocyte counts [40]. Additional laboratory findings seen commonly include polyclonal hypergammaglobulinemia, auto-antibodies to platelets, neutrophils and red blood cells, elevation of serum vitamin B12, soluble

FASL, soluble CD25 (IL2R- α) and cytokines including IL-10, IL-18 and BAFF ([27,28^{***}] and unpublished data). Antinuclear antibodies and rheumatoid factor are not commonly seen in ALPS.

Autoimmune lymphoproliferative syndrome patients have enlarged lymph nodes with follicular hyperplasia and marked paracortical expansion involving immunoblasts and plasma cells [41]. In addition, the paracortical areas demonstrate large numbers of infiltrating $\alpha\beta$ -DNT cells. The DNT cells in the lymph nodes and peripheral blood have the same immunophenotypic characteristics, including HLA-DR, CD57 and CD45RA expression [41,42].

Diagnostic approach

The diagnostic criteria required for a definitive diagnosis of ALPS include nonmalignant lymphocyte accumulation, increased levels of $\alpha\beta$ -DNT cells and either defective in-vitro FAS-mediated lymphocyte apoptosis or a gene mutation known to be associated with ALPS (listed above under the 'Genetics and pathophysiology' subsection) [29]. During the diagnostic work-up, one should first exclude cancer and infectious diseases as the cause of the lymphoaccumulation before proceeding with the investigation of possible ALPS. An initial screening with flow cytometric evaluation of peripheral blood lymphocytes is necessary to detect the presence of increased levels of $\alpha\beta$ -DNT cells. As noted previously, there is often a B-cell lymphocytosis, the majority of which co-express CD5 but do not express CD27 as well as decreased levels of CD4⁺CD25⁺ T cells [40]. Next, it is important to establish the site of the genetic defect. Mutations in *FAS* represent the most common defect, and genomic DNA sequencing of this gene should be the starting point. However, if this is normal in the setting of an elevation in $\alpha\beta$ -DNT cells and increased levels of one or more biomarkers (sFASL, IL-10, vitamin B12), then sequencing genomic DNA obtained from highly purified DNT T cells would be the next step to evaluate for a somatic *FAS* mutation. When these studies are unrevealing, it is appropriate to then sequence the genes encoding FASL and caspase 10. Despite this approach there remain ALPS patients without defined genetic abnormalities with proven defective lymphocyte apoptosis *in vitro* (i.e. ALPS-U). In the presence of the two required criteria but in ability to obtain in-vitro apoptosis testing or genetic studies, a provisional diagnosis of 'probable ALPS' can be made if one of the secondary accessory criterion is present [29]. This means that the patient must be treated and monitored for development of lymphoma as is done in patients with ALPS until a definitive diagnosis can be determined.

Treatment

The lymphoid expansion typically diminishes with age; thus therapy directed at this feature of ALPS is generally not necessary [38]. Splenomegaly is often associated with hypersplenism and splenectomy has been commonly used in ALPS patients. However, owing to a high incidence of serious bacterial infections with resultant mortality postsplenectomy, this is discouraged unless absolutely necessary [16^{*},38]. Autoimmune cytopenias typically respond to corticosteroids, and this therapy is also associated with a decrease in lymphadenopathy; unfortunately the latter rapidly reappears after discontinuation of corticosteroids. ITP exacerbations are not uncommon and these may become resistant to conventional therapy in some patients. ALPS thrombocytopenia often does not respond to intravenous immunoglobulin therapy in contrast to most childhood ITP cases. Accumulating data suggest that mycophenolate mofetil can be useful in the treatment of ALPS patients with unresponsive cytopenias [43^{***},44–46]. In addition, the use of rapamycin is currently being evaluated in ALPS based on preliminary data demonstrating resolution of cytopenias, decrease in lymphadenopathy and diminished levels of $\alpha\beta$ -DNT cells while on therapy [47,48]. The potential for lymphoma appears to be a lifelong risk in patients with germline *FAS* mutations (the risk is not known in other categories of ALPS) that warrants careful monitoring; to date it appears that the treatment of lymphomas in ALPS patients appears to follow standard response patterns.

CASPASE-8 DEFICIENCY SYNDROME

Two siblings with homozygous mutations in *CASP8* were described in 2002 [49]. These patients presented findings suggestive of ALPS including splenomegaly, lymphadenopathy, defective FAS apoptosis *in vitro* and lymphocytosis, but had only minimally elevated $\alpha\beta$ -DNTs and no clinical autoimmune disease [43^{***}]. However, unlike other ALPS patients, these individuals had clinical signs of a mild combined immunodeficiency, with recurrent sinopulmonary and mucocutaneous herpes simplex infections together with mild hypogammaglobulinemia (Fig. 1). In-vitro evaluation revealed defective T, B and natural killer (NK)-cell activation, findings that were later explained by the demonstration of the importance of caspase-8 for the activation of nuclear factor (NF)- κ B downstream following cell surface receptor activation [50]. More recently, two additional *CASP8*-deficient patients were identified, distantly related to the original family, whose disease manifested only in adulthood with splenomegaly, recurrent sinopulmonary infections, warts,

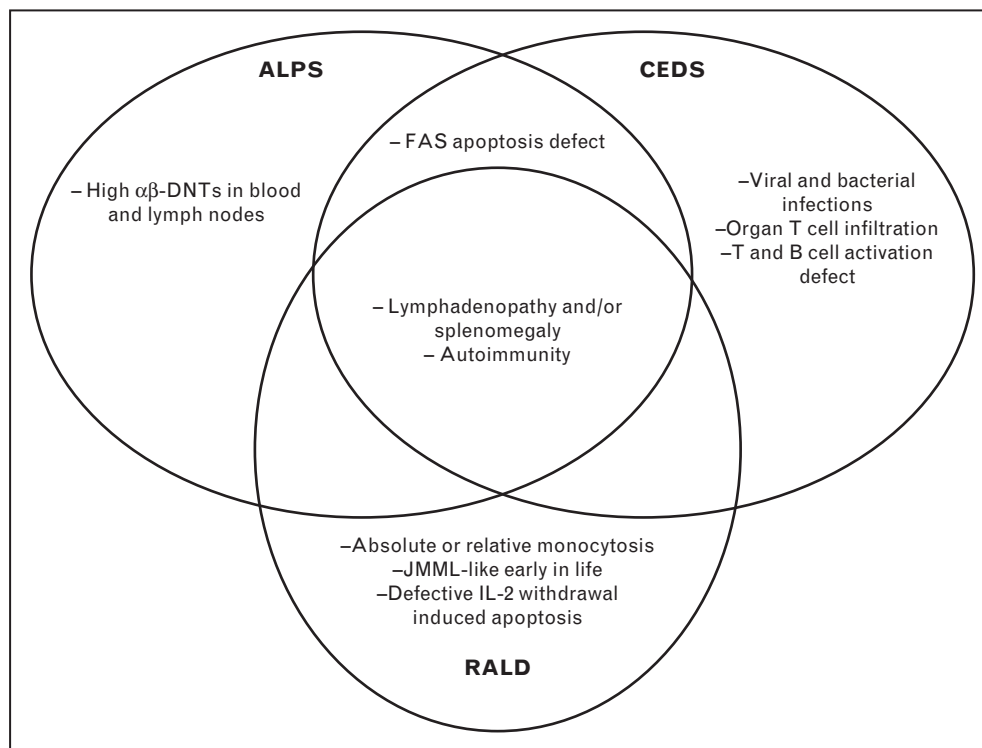


FIGURE 1. Venn diagram demonstrating the relationships between ALPS, RALD and CEDS. ALPS, autoimmune lymphoproliferative syndrome; CEDS, caspase-8 deficiency disorder; JMML, juvenile myelomonocytic leukemia; RALD, RAS-associated autoimmune leukoproliferative disorder.

molluscum contagiosum and lymphocytic infiltration of multiple organs including brain, liver and lungs (Oliveira and Rosenzweig, personal communication). Of note, $\alpha\beta$ -DNTs were within normal ranges in both patients and these clinical findings appear to expand the clinical spectrum of caspase-8 deficiency syndrome (CEDS).

RAS-ASSOCIATED AUTOIMMUNE LEUKOPROLIFERATIVE DISORDER

In 2007, while investigating the genetic basis of disease in a group of patients with features of ALPS but without mutation in known genes, the authors discovered one patient with a somatic mutation in the gene *NRAS* [7]. Although initially classified as ALPS without a genetic basis, the patient had atypical features such as a history of significant leukocytosis early in life, persistent monocytosis and a low level of $\alpha\beta$ -DNTs, which did not infiltrate lymph nodes. Later, additional patients with an ALPS-like disease and mutations in *NRAS* and *KRAS* were identified, and the disease was named RAS-associated autoimmune leukoproliferative disorder (RALD) [6[•],7,8].

Clinical and laboratory findings

The patients with RALD described to date have several overlapping clinical and laboratory features

with ALPS (Fig. 1). They were diagnosed at ages varying from 1 to 47 years of life, and presented with some degree of peripheral lymphadenopathy as well as splenomegaly and autoimmune phenomena, including hemolytic anemia, immune thrombocytopenia and neutropenia. In some patients, a history of recurrent mild upper and lower respiratory tract infections could be elicited, but this was not consistent in all patients evaluated [6[•]]. Inconsistent with ALPS was a history of transient or persistent elevation in granulocytes and monocytes. Some RALD patients had a clinical and laboratory phenotype very similar to juvenile myelomonocytic leukemia (JMML) early in life, with marked hepatosplenomegaly and monocytosis. However, unlike patients with JMML, the monocytosis improved spontaneously (but did not disappear).

The immunophenotypic pattern in these patients displayed mild to no elevation in $\alpha\beta$ -DNT cells and a relative expansion of B cells. Total lymphocyte numbers may be normal to modestly decreased. In contrast, absolute or relative monocytosis was noted in all patients. Auto-antibodies are typically detected including, ANA, rheumatoid factor as well as antiphospholipid, anticardiolipin antiplatelet, antineutrophil and/or antired cell antibodies [6[•],7,8]. Unlike patients with ALPS, the biomarkers and in-vitro FAS-induced apoptosis

are also normal. In contrast, the T cells are resistant to IL-2 withdrawal-induced cell death pointing to a fundamentally different apoptotic defect in RALD [6[■],7,8]. The histopathological findings include nonspecific polyclonal plasmacytosis with reactive secondary follicles, but without the typical paracortical expansion by $\alpha\beta$ -DNT cells seen in ALPS. Given the small number of patients diagnosed to date, it is not known if these patients are at increased risk for hematological malignancy. In summary, RALD should be suspected in a patient with splenomegaly with or without lymphadenopathy associated with blood cell-directed autoimmunity and relative or absolute monocytosis in whom a malignancy or infection has been ruled out.

Genetics and pathophysiology

All patients with RALD described to date harbor somatic, gain-of-function mutations in *KRAS* or *NRAS* [6[■],7,8]. As expected with somatic mutations the family history is typically unrevealing and the mutations are confined to blood cells. Somatic activating mutations in RAS have previously not been described in nontumoral syndromes, but are present in up to 30% of all human cancers [51]. Germline RAS pathway mutations have been described as causing the related Costello (HRAS), Noonan (PTPN11, KRAS, SOS1, NRAS) and cardiofaciocutaneous syndromes (KRAS, BRAF, MEK1, and MEK) [52].

The *NRAS* and *KRAS* mutations seen in RALD patients are heterozygous and gain-of-function, including p.G13D and p.G12V, similar to those described in tumors. These mutations disrupt the interaction of RAS with the GTPase-activating proteins (GAPs), diminishing its GTPase activity by over 300-fold, locking the molecule in an 'on' position [53,54]. This permanent activation state increases cell signaling through several pathways including the ERK pathway, inducing the phosphorylation and destruction of the proapoptotic protein BIM [6[■],7]. Consequently, the cells become resistant to certain kinds of apoptotic stimuli, such as growth factor (IL-2) withdrawal. Additionally, the persistent ERK signaling decreases the intracellular levels of negative inhibitors of the cell cycle, namely p27^{kip1}, allowing increased proliferation in the face of limiting IL-2 levels [6[■]]. Given the multiple antibody-mediated autoimmune manifestations seen in these patients, it is interesting to note that recent work has suggested that adequate RAS signaling is important for B-cell selection [55].

CONCLUSION

The results of 20 years of studies in patients with ALPS have established the genetic basis for the

majority of these patients and have provided the first direct evidence for a human autoimmune disease linked to a genetic defect in lymphocyte apoptosis. The restricted nature of autoimmunity in ALPS should help to better clarify immunoregulatory pathways controlling autoreactivity to hematopoietic elements, as well as the role of FAS-mediated apoptosis as a tumor-suppressor element associated with the development of B-cell lymphomas. ALPS represents an 'experiment of nature' involving lymphocyte apoptosis that continues to provide important insights into the role of apoptosis in controlling lymphocyte expansion and homeostasis, as well as providing opportunities for investigating therapeutic approaches in managing disease-associated manifestations. The identification of RALD provides another chapter in our understanding of apoptosis as a complex scheme involved in lymphocyte (and leukocyte) homeostasis as well as in the control of autoreactivity.

Acknowledgements

None.

Conflicts of interest

The research was supported by the Intramural Research Program of the NIH.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Bidere N, Su HC, Lenardo MJ. Genetic disorders of programmed cell death in the immune system. *Annu Rev Immunol* 2006; 24:321–352.
 2. Fisher GH, Rosenberg FJ, Straus SE, *et al.* Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 1995; 81:935–946.
 3. Rieux-Laucat F, Le Deist F, Hivroz C, *et al.* Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* 1995; 268:1347–1349.
 4. Lenardo M, Chan KM, Hornung F, *et al.* Mature T lymphocyte apoptosis: immune regulation in a dynamic and unpredictable antigenic environment. *Annu Rev Immunol* 1999; 17:221–253.
 5. Jagger AL, Evans HG, Walter GJ, *et al.* FAS/FAS-L dependent killing of activated human monocytes and macrophages by CD4+CD25- responder T cells, but not CD4+CD25+ regulatory T cells. *J Autoimmun* 2012; 38:29–38.
 6. Niemela JE, Lu L, Fleisher TA, *et al.* Somatic KRAS mutations associated with ■ a human nonmalignant syndrome of autoimmunity and abnormal leukocyte homeostasis. *Blood* 2011; 117:2883–2886.
- Initial description of KRAS mutations associated with RALD.
7. Oliveira JB, Bidere N, Niemela JE, *et al.* NRAS mutation causes a human autoimmune lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* 2007; 104:8953–8958.
 8. Takagi M, Shinoda K, Piao J, *et al.* Autoimmune lymphoproliferative syndrome-like disease with somatic KRAS mutation. *Blood* 2011; 117:2887–2890.
 9. Kono DH, Theofilopoulos AN. Genetics of systemic autoimmunity in mouse models of lupus. *Int Rev Immunol* 2000; 19:367–387.
 10. Watanabe-Fukunaga R, Brannan CI, Copeland NG, *et al.* Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 1992; 356:314–317.

11. Takahashi T, Tanaka M, Brannan CI, *et al.* Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* 1994; 76:969–976.
12. Canale VC, Smith CH. Chronic lymphadenopathy simulating malignant lymphoma. *J Pediatr* 1967; 70:891–899.
13. Sneller MC, Straus SE, Jaffe ES, *et al.* A novel lymphoproliferative/autoimmune syndrome resembling murine *lpr/gld* disease. *J Clin Invest* 1992; 90:334–341.
14. Drappa J, Vaishnaw AK, Sullivan KE, *et al.* Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. *N Engl J Med* 1996; 335:1643–1649.
15. Behrmann I, Walczak H, Krammer PH. Structure of the human APO-1 gene. *Eur J Immunol* 1994; 24:3057–3062.
16. Neven B, Magerus-Chatinet A, Florkin B, *et al.* A survey of 90 patients with autoimmune lymphoproliferative syndrome related to TNFRSF6 mutation. *Blood* 2011; 118:4798–4807.
- An excellent summary of the clinical features of ALPS-FAS patients.
17. Kuehn HS, Caminha I, Niemela JE, *et al.* FAS haploinsufficiency is a common disease mechanism in the human autoimmune lymphoproliferative syndrome. *J Immunol* 2011; 186:6035–6043.
- Study identifying the disease-producing mechanisms in the majority of ALPS patients with extracellular FAS mutations.
18. Siegel RM, Frederiksen JK, Zacharias DA, *et al.* Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 2000; 288:2354–2357.
19. Benkerrou M, Le Deist F, de Villartay JP, *et al.* Correction of Fas (CD95) deficiency by haploidentical bone marrow transplantation. *Eur J Immunol* 1997; 27:2043–2047.
20. Sleight BJ, Prasad VS, DeLaat C, *et al.* Correction of autoimmune lymphoproliferative syndrome by bone marrow transplantation. *Bone Marrow Transplant* 1998; 22:375–380.
21. Wu J, Wilson J, He J, *et al.* Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J Clin Invest* 1996; 98: 1107–1113.
22. Cerutti E, Campagnoli MF, Ferretti M, *et al.* Co-inherited mutations of Fas and caspase-10 in development of the autoimmune lymphoproliferative syndrome. *BMC Immunol* 2007; 8:28.
23. Bi LL, Pan G, Atkinson TP, *et al.* Dominant inhibition of Fas ligand-mediated apoptosis due to a heterozygous mutation associated with autoimmune lymphoproliferative syndrome (ALPS) type Ib. *BMC Med Genet* 2007; 8:41.
24. Wang J, Zheng L, Lobito A, *et al.* Inherited human Caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. *Cell* 1999; 98:47–58.
25. Holzelova E, Vonarbourg C, Stolzenberg MC, *et al.* Autoimmune lymphoproliferative syndrome with somatic Fas mutations. *N Engl J Med* 2004; 351:1409–1418.
26. Dowdell KC, Niemela JE, Price S, *et al.* Somatic FAS mutations are common in patients with genetically undefined autoimmune lymphoproliferative syndrome. *Blood* 2010; 115:5164–5169.
- A summary of the frequency of somatic FAS mutations in a large cohort of ALPS patients.
27. Magerus-Chatinet A, Stolzenberg MC, Loffredo MS, *et al.* FAS-L, IL-10, and double-negative CD4⁺ CD8⁺ TCR alpha/beta⁺ T cells are reliable markers of autoimmune lymphoproliferative syndrome (ALPS) associated with FAS loss of function. *Blood* 2009; 113:3027–3030.
28. Caminha I, Fleisher TA, Homung RL, *et al.* Using biomarkers to predict the presence of FAS mutations in patients with features of the autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol* 2010; 125:946–949; e946.
- Study linking the presence of specific biomarkers to ALPS associated with mutations in FAS.
29. Oliveira JB, Bleesing JJ, Dianzani U, *et al.* Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood* 2010; 116:e35–e40.
30. Infante AJ, Britton HA, DeNapoli T, *et al.* The clinical spectrum in a large kindred with autoimmune lymphoproliferative syndrome caused by a Fas mutation that impairs lymphocyte apoptosis. *J Pediatr* 1998; 133:629–633.
31. Rieux-Laucat F, Blachere S, Danielan S, *et al.* Lymphoproliferative syndrome with autoimmunity: a possible genetic basis for dominant expression of the clinical manifestations. *Blood* 1999; 94:2575–2582.
32. Jackson CE, Fischer RE, Hsu AP, *et al.* Autoimmune lymphoproliferative syndrome with defective Fas: genotype influences penetrance. *Am J Hum Genet* 1999; 64:1002–1014.
33. Hsu AP, Dowdell KC, Davis J, *et al.* Autoimmune lymphoproliferative syndrome due to FAS mutations outside the signal-transducing death domain: molecular mechanisms and clinical penetrance. *Genet Med* 2012; 14:81–89.
34. Magerus-Chatinet A, Neven B, Stolzenberg MC, *et al.* Onset of autoimmune lymphoproliferative syndrome (ALPS) in humans as a consequence of genetic defect accumulation. *J Clin Invest* 2011; 121:106–112.
35. Bleesing JJ, Straus SE, Fleisher TA. Autoimmune lymphoproliferative syndrome. A human disorder of abnormal lymphocyte survival. *Pediatr Clin North Am* 2000; 47:1291–1310.
36. Sneller MC, Wang J, Dale JK, *et al.* Clinical, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. *Blood* 1997; 89:1341–1348.
37. Kwon SW, Procter J, Dale JK, *et al.* Neutrophil and platelet antibodies in autoimmune lymphoproliferative syndrome. *Vox Sang* 2003; 85:307–312.
38. Rao VK, Oliveira JB. How I treat autoimmune lymphoproliferative syndrome. *Blood* 2011; 118:5741–5751.
39. Straus SE, Jaffe ES, Puck JM, *et al.* The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. *Blood* 2001; 98:194–200.
40. Bleesing JJ, Brown MR, Straus SE, *et al.* Immunophenotypic profiles in families with autoimmune lymphoproliferative syndrome. *Blood* 2001; 98:2466–2473.
41. Lim MS, Straus SE, Dale JK, *et al.* Pathological findings in human autoimmune lymphoproliferative syndrome. *Am J Pathol* 1998; 153:1541–1550.
42. Bleesing JJ, Brown MR, Novicio C, *et al.* A composite picture of TcR alpha/beta(+) CD4(+) CD8(+) T Cells (alpha/beta-DNTCs) in humans with autoimmune lymphoproliferative syndrome. *Clin Immunol* 2002; 104:21–30.
43. Teachey DT. New advances in the diagnosis and treatment of autoimmune lymphoproliferative syndrome. *Curr Opin Pediatr* 2012; 24:1–8.
- An excellent discussion of various therapeutic options in ALPS.
44. Arora S, Singh N, Chaudhary GK, John MJ. Autoimmune lymphoproliferative syndrome: response to mycophenolate mofetil and pyrimethamine/sulfadoxine in a 5-year-old child. *Indian J Hematol Blood Transfus* 2011; 27:101–103.
45. Kossiva L, Theodoridou M, Mostrou G, *et al.* Mycophenolate mofetil as an alternate immunosuppressor for autoimmune lymphoproliferative syndrome. *J Pediatr Hematol Oncol* 2006; 28:824–826.
46. Rao VK, Dugan F, Dale JK, *et al.* Use of mycophenolate mofetil for chronic, refractory immune cytopenias in children with autoimmune lymphoproliferative syndrome. *Br J Haematol* 2005; 129:534–538.
47. Janic MD, Brasanac CD, Jankovic JS, *et al.* Rapid regression of lymphadenopathy upon rapamycin treatment in a child with autoimmune lymphoproliferative syndrome. *Pediatr Blood Cancer* 2009; 53:1117–1119.
48. Teachey DT, Grupp SA, Brown VI. Mammalian target of rapamycin inhibitors and their potential role in therapy in leukaemia and other haematological malignancies. *Br J Haematol* 2009; 145:569–580.
49. Chun HJ, Zheng L, Ahmad M, *et al.* Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature* 2002; 419:395–399.
50. Su H, Bidere N, Zheng L, *et al.* Requirement for caspase-8 in NF-kappaB activation by antigen receptor. *Science* 2005; 307:1465–1468.
51. Barbacid M. Ras genes. *Annu Rev Biochem* 1987; 56:779–827.
52. Zenker M. Clinical manifestations of mutations in RAS and related intracellular signal transduction factors. *Curr Opin Pediatr* 2011; 23:443–451.
53. Tong LA, de Vos AM, Milburn MV, *et al.* Structural differences between a ras oncogene protein and the normal protein. *Nature* 1989; 337:90–93.
54. de Vos AM, Tong L, Milburn MV, *et al.* Three-dimensional structure of an oncogene protein: catalytic domain of human c-H-ras p21. *Science* 1988; 239:888–893.
55. Limnander A, Weiss A. Ca-dependent Ras/Erk signaling mediates negative selection of autoreactive B cells. *Small Gtpases* 2011; 2:282–288.