

## Autoimmune Lymphoproliferative Syndrome\*

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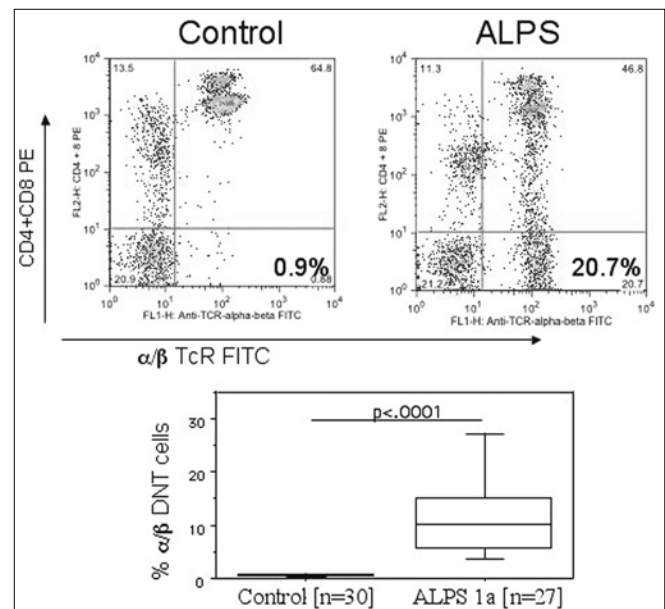
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The autoimmune lymphoproliferative syndrome is a recently described human disorder that affects lymphocyte programmed cell death (apoptosis), resulting in altered immune homeostasis. The hallmark feature of this human “experiment of nature” is lymphoproliferation, presenting as non-malignant lymphadenopathy and splenomegaly during childhood [1–3]. This is often accompanied by the development of autoimmunity that primarily affects blood cells. Laboratory findings include an expansion of a normally rare lymphoid subpopulation of T cells that do not express CD4 or CD8 (double negative T cells) but express the alpha/beta form of the T cell antigen receptor [Figure 1] [4]. In addition, ALPS patients often have polyclonal increases in serum immunoglobulin levels as well as increased serum interleukin-10 levels [5]. The clinical course typically involves unexplained and persistent lymphadenopathy presenting early in childhood followed by the appearance of autoimmune disease, most frequently autoimmune hemolytic anemia and/or autoimmune thrombocytopenia. Immune neutropenia may also be seen, while other autoimmune disorders present only infrequently. Autoimmunity can occur throughout life although the initial presentation is most common during childhood. While conventional therapy has been the mainstay for managing the immune cytopenias, newer treatment approaches have been used as a splenectomy-sparing alternative due to the infectious risk that follows this surgical procedure [6]. Lymphoid malignancy is also more common in ALPS and can appear at any age but is most frequent during adulthood. The clinical evolution of ALPS from initial presentation varies, although not infrequently the lymphadenopathy becomes less prominent following adolescence. Patients require regular monitoring due to the lifetime risk of lymphoma development. The clinical and laboratory observations in these patients have been complemented by a series of studies documenting the underlying molecular defect in the majority of ALPS patients.

The initial focus on *in vitro* Fas-mediated lymphocyte apoptosis in ALPS was prompted by published studies defining the underlying defects in the *lpr* and *gld* murine models of autoimmunity [8]. These reports identified autosomal recessive mutations in either the gene encoding Fas (*lpr*) or Fas ligand (*gld*), both of which resulted in defective Fas-mediated

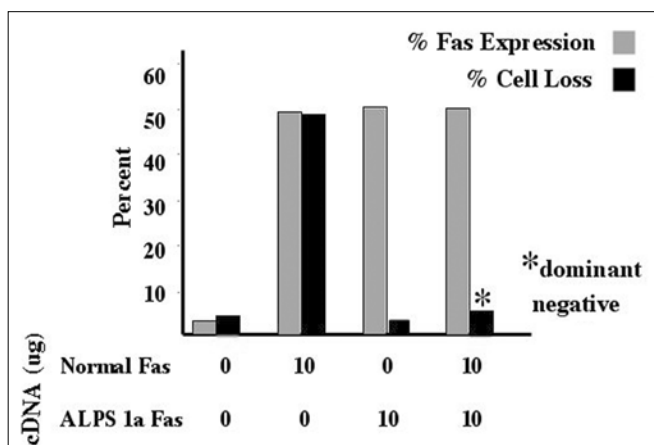
lymphocyte apoptosis [7]. It had also been observed that the severity of disease was dependent on the genetic background of the mice expressing either mutation, suggesting that additional factors impact disease penetrance. Importantly, the hallmark findings in the *lpr* and *gld* mice include an increase in alpha/beta DNT cells associated with massive, non-malignant lymphadenopathy and an increased risk of developing a B cell lineage tumor (plasmacytoma) as well as systemic lupus erythematosus-like autoimmunity. Recognition that these two murine models had many similarities with the clinical picture in ALPS prompted evaluation of the Fas-mediated death pathway in the patients. These studies noted that the human disorder was associated with defective *in vitro* Fas-mediated lymphocyte apoptosis in both activated T cells and Epstein-Barr virus-transformed B cell lines. These findings, when linked to the mutation data in the *lpr* and *gld* mice, prompted the evaluation of the genes encoding Fas and Fas



**Figure 1.** Flow cytometry contour plots of a control subject and a representative ALPS patient plotting CD4 and CD8 expression (Y axis) with alpha/beta TcR expression (X axis). The percentage of CD4/CD8 double negative T (DNT) cells are shown in the right lower quadrant of each contour plot. The cumulative data for percent alpha/beta DNT cells from 30 controls and 27 ALPS 1a patients are shown in the lower plot.

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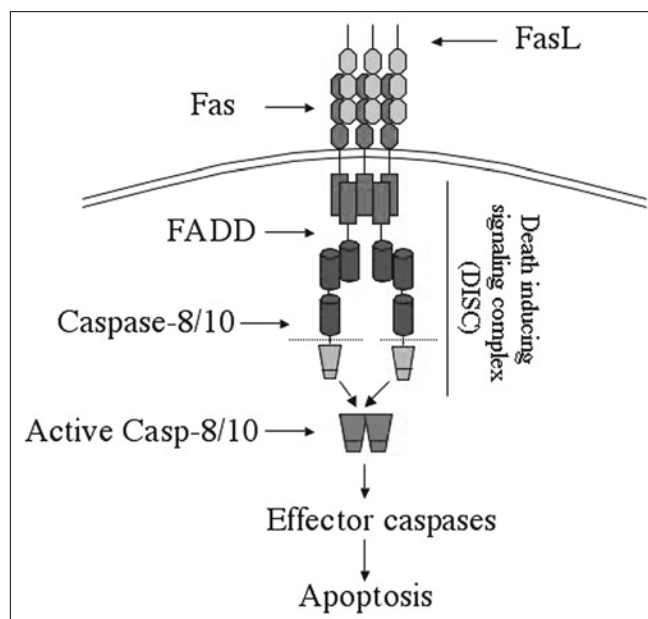
ALPS = autoimmune lymphoproliferative syndrome  
DNT = double negative T cells



**Figure 2.** Experiments in which either cDNA obtained from a normal control and an ALPS type 1a patient was transfected into a murine cell line that did not express Fas. Mock transfected cells neither expressed Fas nor were induced to undergo apoptosis by addition of a cross-linking anti-Fas monoclonal antibody. Transfection with control Fas cDNA resulted in both Fas expression and apoptosis in response to anti-Fas antibody, while transfection with ALPS 1a cDNA resulted in virtually no cell death with anti-Fas addition despite equivalent Fas expression. Co-transfection with normal and patient Fas demonstrated Fas expression but no cell death, establishing dominant negative inhibition by the mutant (ALPS) Fas.

ligand, work that identified mutations in the gene encoding Fas (*TNFRSF6*) in a significant proportion of ALPS patients [8–10]. However, unlike the *lpr* mouse, the ALPS patients had mutations that affected only one allele. Evaluation of a large number of ALPS patients revealed that mutations can be found throughout the gene, although the majority involves regions encoding the intracellular portion of the receptor and in particular the death domain encoded by exon 9. These findings established a major distinction between the human disorder and the murine model beyond the different patterns of autoimmunity; namely, ALPS appeared to be inherited as an autosomal dominant disorder whereas the murine disorder followed an autosomal recessive pattern.

The causal relationship between heterozygous Fas mutations found in ALPS patients and defective lymphocyte apoptosis was obtained via a series of experiments in which cells that do not express Fas were transfected with normal (wild-type) Fas cDNA, ALPS patient (mutant) Fas cDNA, or a combination of the two [8]. As seen in Figure 2, transfection with wild-type Fas resulted in both Fas expression and cell death induced by experimental Fas cross-linking. However, when the cells were transfected with mutant Fas, cross-linking failed to induce cell death despite equivalent levels of Fas expression. Thus, the ALPS mutations studied resulted in cell surface protein expression of the mutant receptor but it was non-functional. To prove that mutant Fas acts as a dominant negative inhibitor, co-transfection experiments were performed demonstrating that despite equivalent Fas expression the combination of normal and patient Fas resulted in marked inhibition of Fas-mediated apoptosis. Taken together, these experiments demonstrate that one defective al-



**Figure 3.** Scheme of Fas activation induced when a homotrimeric Fas receptor is bound by Fas ligand (also assembled as a homotrimer). This results in FADD binding to the death domains of the Fas homotrimer, producing the death-inducing signaling complex (DISC) that activates either procaspase 8 or 10 generating the active enzyme. This last step initiates the apoptotic cascade that ultimately results in death of the cell. This process can be mimicked *in vitro* by cross-linking Fas homotrimers using specific monoclonal antibodies to Fas.

lele affecting the death domain was sufficient to interfere with Fas function.

The next focus was to establish the underlying mechanism whereby heterozygous Fas mutations affecting the death domain result in the dominant negative inhibition of Fas-mediated apoptosis. This work was developed in the setting of studies that established that signaling via Fas requires pre-assembly of a homotrimeric Fas receptor prior to the binding of its specific ligand [Figure 3] [11]. In ALPS patients with a heterozygous mutation affecting the death domain, normal assembly of Fas trimers would generate seven out of eight trimers containing at least one mutant protein. Experiments by Martin et al. [12] demonstrated that one mutant death domain within the homotrimeric Fas receptor was sufficient to inhibit the normal binding of the critical homotypic intracellular protein FADD (Fas-associated death domain). They went on to show that interference with FADD binding (in the context of heterozygous death domain mutations) prevents the assembly of the death-inducing signaling complex, blocks further activation of the apoptotic cascade and prevents apoptosis.

A subgroup of ALPS patients was found to have Fas mutations affecting the extracellular domains. These occurred at a lower frequency than those affecting the intracellular domains, and evaluation of family pedigrees in this subgroup identified that the extracellular mutations were associated with lower pen-

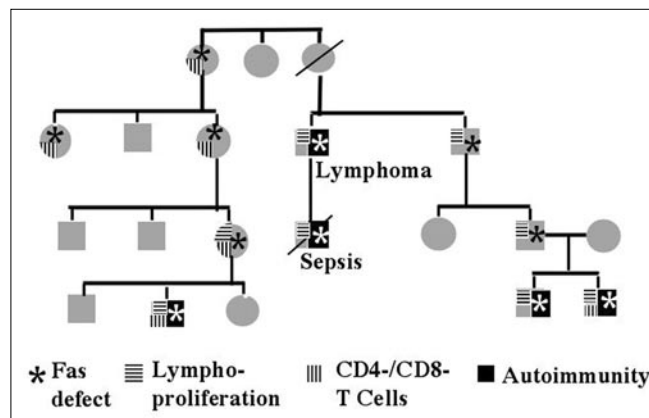
FADD = Fas-associated death domain

etrance [13]. In addition, this subgroup of ALPS patients demonstrated a less severe *in vitro* apoptotic defect [14], and more recently the majority of extracellular mutations was found to interfere with Fas function via haploinsufficiency or modified ligand binding rather than via direct interference with Fas signaling (Hsu and Puck, unpublished observations).

With the evolving understanding of clinical and molecular features of ALPS, a set of diagnostic criteria for ALPS was established, and these include non-malignant lymphadenopathy and/or splenomegaly, peripheral expansion of alpha/beta DNT cells, and abnormal *in vitro* lymphocyte apoptosis. As more patients were identified, several were found to fulfill the diagnostic triad but did not have mutations in the gene encoding Fas. This led to mutation analysis directed at other genes involved in the Fas pathway, studies that identified a limited number of patients with defects in the genes encoding either Fas ligand or caspase 10 [15,16]. The latter cases were found to have dominant negative interfering mutations that inhibit the generation of active caspase 10, preventing the intracellular apoptotic cascade (Zhu and Puck, unpublished observations). Taken together, these findings led to the ALPS categorization scheme: ALPS type 1a due to mutations affecting Fas, ALPS type 1b related to mutations in Fas ligand, and ALPS type 2 associated with mutations in caspase 10. However, applying the diagnostic triad noted above also identified a group of patients with essentially identical clinical findings but without a demonstrable mutation in the genes noted above. Thus, although the genetic basis for ALPS has been clarified in the majority of patients, there remains a subgroup fulfilling the diagnostic triad without an identified genetic lesion (ALPS type 3). Studies in this group of patients are focused on identifying the underlying genetic basis of disease; recently a number of ALPS type 3 patients was described with somatic (but not germline) mutations in the gene encoding Fas [17]. In addition, there are also patients who have the same clinical findings but do not have a defect in Fas-mediated lymphocyte apoptosis and will be referred to as "ALPS-like" in this review.

The patients with "ALPS-like" disease represent a fascinating group who require additional studies to identify the underlying apoptotic defect(s) responsible for their disease. Our team has initiated studies in these patients and recently evaluated one patient who appears to have an alternative apoptotic defect that does not involve the Fas pathway (Oliveira, Lenardo and Fleisher, unpublished data). It seems very likely that these patients will provide additional insights into immune homeostasis and critical processes involved in the development of certain types of autoimmunity. Likewise, the genetic lesions underlying disease in patients with ALPS type 3 remain undefined, and in-depth studies of these individuals should provide further insight into the other molecules and regulatory processes that participate in the Fas apoptotic pathway.

ALPS type 1a is the most frequent presentation, and studies of family pedigrees have provided additional information regarding this disorder. As seen in Figure 4, inheritance of the same mutation within four generations in one family resulted in



**Figure 4.** A family pedigree of ALPS type 1a showing individuals from four generations within this one family. Among those who inherited the same mutant Fas gene there was substantial difference in the clinical phenotypes, ranging from a single finding including increased alpha/beta DNT cells or lymphoproliferation to clinical ALPS.

significantly different phenotypes – ranging from limited clinical findings to ALPS. This underscores the concept that clinical disease in ALPS is a product of the mutant Fas gene plus one or more undefined genetic factors and/or environmental effects. This observation concurs with findings in the *lpr* and *gld* murine models where strain differences result in significant variation in the severity of autoimmunity. It is also clear that having two defective Fas alleles (either homozygous or compound heterozygous) results in a more severe disease that presents during the neonatal period and has required far more aggressive therapeutic approaches including bone marrow transplantation [18,19].

Another clinical observation emerged as more patients were identified – a high frequency of lymphoma among both ALPS type 1a probands and their mutation-positive family members [20]. Review of these cases revealed that most were B cell lineage tumors with varying histologic types, and formal assessment of the relative risk for lymphoma yielded a relative risk for Hodgkin's lymphoma of 51-fold and for non-Hodgkin's lymphoma of 14-fold [20,21]. Tumor tissue from one patient was available for further study and the malignant cells were found to carry the same Fas mutation previously found in genomic DNA [20]. Overall, the lymphomas in ALPS have responded to conventional therapy and appear to behave like the equivalent tumor in non-ALPS patients. One ALPS patient in this series developed two different lymphomas – the first was successfully treated with chemotherapy while the second presented years later and proved ultimately fatal. Importantly, virtually all lymphoma cases have been found in ALPS type 1a patients with mutations affecting the death domain of Fas. These defects result in the greatest suppression in Fas-mediated lymphocyte apoptosis and were previously shown to have the highest degree of penetrance in family studies. This suggests that the magnitude of the apoptotic defect is linked to the risk of developing a lymphoid malignancy.

The ALPS story is approximately 15 years old and has evolved as a product of successfully integrating lessons at

the “bedside” with work at the “bench” to more clearly define this human condition. This effort has not only documented the clinical phenotype of ALPS and the underlying molecular mechanisms but has also provided valuable information on the function of the Fas pathway in immune homeostasis. These studies have been central in clarifying the role of Fas in the control of lymphoid expansion, the development of specific forms of autoimmunity, and the inhibition of lymphoid malignancy. However, the story is far from complete and the host of unanswered questions will only be addressed by the continued investigation into patients with ALPS and “ALPS-like” conditions.

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