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The autoimmune lymphoproliferative syndrome: an experiment of nature involving lymphocyte apoptosis

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Abstract Autoimmune lymphproliferative syndrome (ALPS) is a human disorder that has been characterized in the past two decades at both a functional and a genetic level. The underlying basis for this disorder is a defect in lymphocyte apoptosis that alters immune homeostasis resulting in an expansion of a normally rare circulating lymphocyte, the alpha beta double negative T cell. The abnormality in Fas mediated apoptosis underlying ALPS serves as a risk factor for autoimmunity involving blood cells and the development of lymphoma. There remain patients with a diagnosis of ALPS but without a defined genetic defect and current investigations are focusing on fully characterizing this patient subgroup.

Keywords Apoptosis · Autoimmunity · Fas · Immune homeostasis

The model of using the clinical findings associated with "experiments of nature" as the starting point to define the underlying mechanisms of human disease was a pillar in the approach to clinical investigation advocated by Dr. Robert A. Good. The description and elucidation of the underlying basis of the autoimmune lymphoproliferative syndrome (ALPS) followed the paradigm that Dr. Good advocated throughout his brilliant career. This human disease represents the first defined human defect in cell death (apoptosis) producing a marked alteration in immune homeostasis that presents as non-malignant lymphadenopathy and splenomegaly during childhood [1–3]. This is often accompanied by the development of autoimmunity that primarily affects blood cell together with an increased risk for developing lymphoma. The hallmark laboratory finding is an expansion of a normally rare lymphoid subpopulation of T-cells that do not express CD4 or CD8 (double negative T [DNT]-cells) but do express the alpha-beta form of the T-cell antigen receptor (Fig. 1) [4]. The expansion in alpha-beta DNT-cells is often accompanied by a

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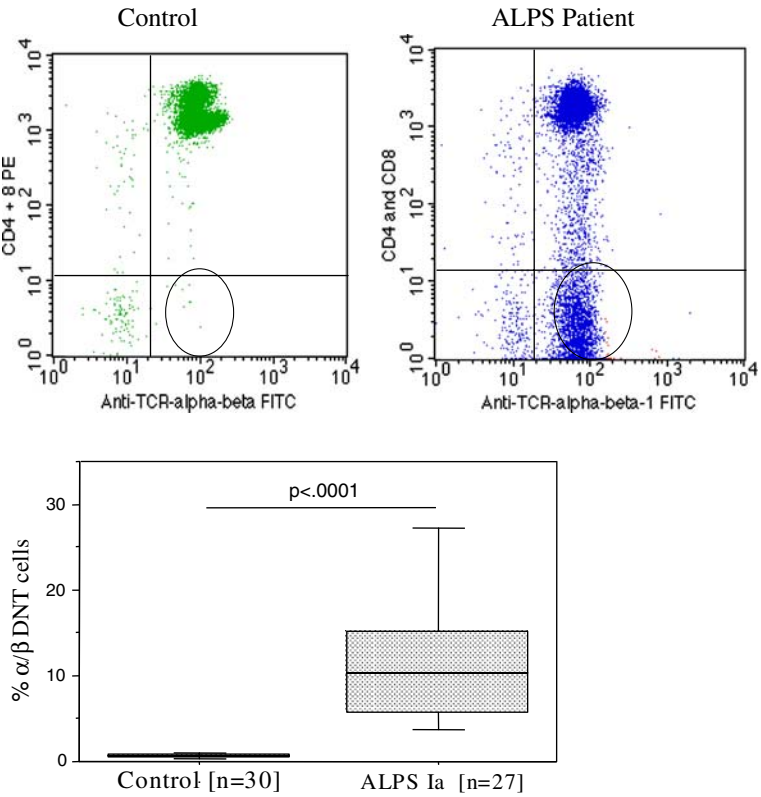

Fig. 1 Dot plot demonstrating the marked expansion in alpha beta double negative T (DNT)-cells (shown in the ovals) of an ALPS patient compared to a control. The lower panel shows the cumulative data of alpha beta DNT cells for 27 ALPS type 1a patients compared to controls [11]

polyclonal increase in serum immunoglobulin levels as well as an increase in serum IL-10 levels [5].

The clinical course involves persistence of the lymphadenopathy throughout childhood often followed by the appearance of autoimmunity that most often presents as autoimmune hemolytic anemia and/or autoimmune thrombocytopenia. Immune neutropenia may also be seen, while other autoimmune disorders present only infrequently. The development of autoimmunity is most common during childhood, although it can occur throughout life. Lymphoid malignancy can also appear throughout life, although it is most frequent during adulthood. The clinical course of ALPS varies, however, the lymphadenopathy usually becomes less prominent following adolescence. The risk of lymphoma appears to be lifelong such that careful follow-up of ALPS patients is critical. The clinical and laboratory observations in the initial group of these patients provided the impetus for a series of experimental studies that documented the underlying molecular defect in the majority of ALPS patients.

The observation that defects in the Fas pathway was responsible for the findings in the *lpr* and *gld* murine models of autoimmunity suggested that a similar line of investigation would be potentially useful in patients with ALPS [6]. This was due to the overlapping characteristic findings between the *lpr* and *gld* mice and the human disease including an

increase in alpha-beta DNT-cells associated with non-malignant lymphadenopathy, autoimmunity (SLE like in the mouse) and an increased risk for the development a B-cell lineage tumor (plasmacytoma). The molecular defect in these murine models was found to be autosomal recessive mutations in either the gene encoding Fas (*lpr*) or Fas ligand (*gld*) that produced defective Fas mediated lymphocyte apoptosis [3]. It had also been observed that the severity of disease was dependent on the genetic background of the mice suggesting that additional factors impact disease penetrance. Clearly, the similarities between ALPS and the murine models were striking and this prompted evaluation of the Fas mediated death pathway in the patients. These studies identified that both activated T-cells and EBV transformed B-cell lines from ALPS patients were defective in Fas mediated apoptosis (Fig. 2), but the cells responded normally to other apoptotic signals including irradiation and staurosporine. In view of the in vitro apoptotic defect in lymphocytes from the patients, the gene encoding Fas was sequenced and mutations in *TNFRSF6* (the gene encoding Fas) was found in a significant proportion of ALPS patients [7–9]. However, unlike the *lpr* mouse, there were a variety of mutations in the patients, and these affected only one allele. Despite the distribution of mutations throughout the gene, the majority involved regions encoding the intracellular portion of the receptor and in particular the death domain encoded by exon 9.

The causal relationship between heterozygous Fas mutations found in ALPS patients and defective lymphocyte apoptosis was defined in a series of experiments in which murine cells that do not express human Fas were transfected with normal (wild type) Fas cDNA, patient (mutant) Fas cDNA or a combination of the two [7]. 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 mutant cDNA obtained from ALPS patients induced cell surface protein expression, but it was non-functional. In order to prove that mutant Fas acts as a dominant negative inhibitor, co-transfection experiments were performed demonstrating that the combination of normal and patient Fas resulted in equivalent Fas expression but marked inhibition of Fas mediated apoptosis. Taken together, these experiments demonstrate that one defective allele affecting the death domain was sufficient to interfere with Fas function.

A subgroup of ALPS patients was found to have mutations affecting the extracellular domains of the gene encoding Fas. However, these occurred at a lower frequency than those affecting the intracellular domains and extended family pedigrees in this subgroup

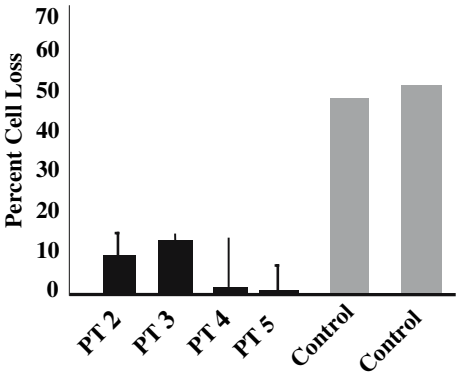

Fig. 2 Fas mediated T-cell apoptosis assay using a cross-linking anti-Fas monoclonal antibody to induce cell death in activated T-cells that were expanded in IL-2. The data presented is the percent cell death in experiments evaluating four ALPS patients and two controls

demonstrated lower penetrance [10]. In addition, this subgroup of ALPS patients demonstrate a less severe in vitro defect of lymphocyte apoptosis [11].

As our understanding of the clinical and molecular features of ALPS developed, a set of diagnostic criteria was established that include: non-malignant lymphadenopathy (and/or splenomegaly); peripheral expansion of alpha-beta DNT-cells; and decreased in vitro lymphocyte apoptosis. As the number of patients who fulfilled the diagnostic triad increased, a subset was identified without a mutation in the gene encoding Fas. This led to analysis directed at other genes involved in the Fas pathway and identified a limited number of patients with defects in the genes encoding either Fas ligand or caspase 10 [12]. The latter cases were found to have a dominant negative interfering mutation that inhibits the generation of active caspase 10 from pro-caspase 10 and blocks the intracellular apoptotic cascade (Zhu and Puck, unpublished observations). These findings led to the ALPS categorization scheme outlined in Table 1 that is generally accepted by other groups involved with studying ALPS patients. However, the diagnostic triad and standard mutation analysis leaves another subgroup of patients, whose clinical and laboratory phenotype are indistinguishable but with no demonstrable mutation identified. Among these are patients with the recently described somatic (but not germline) mutations in the gene encoding Fas [13]. Thus, although the genetic basis for ALPS has been clarified in many patients, there remain a subgroup that fulfills the diagnostic triad without an identified genetic lesion (type 3) and another subgroup of patients who are currently categorized as "ALPS-like" with equivalent clinical characteristics but who do not have the functional in vitro Fas defect.

The patients with "ALPS-like" disease represent an interesting group who require additional studies to identify the underlying defect(s) responsible for their disease. Our group has initiated studies of these patients and has recently evaluated one such patient who appears to have a defect in one aspect of the intrinsic (non-Fas mediated) apoptotic pathway (Oliveira and Fleisher, unpublished data). It seems very likely that these patients will provide additional new insights into immune homeostasis and other critical apoptotic processes involved in the development of certain types of autoimmunity and/or lymphoma. 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 extrinsinc apoptotic pathway mediated by Fas.

ALPS type 1a is the most frequent diagnosis among our patients and studies of extended family pedigrees within this patient group has provided additional lessons regarding the disorder. As seen in Fig. 3, inheritance of the same heterozygous mutation within one family can produce different clinical phenotypes that range from minimal clinical findings

Table 1 Classification of the autoimmune lymphoproliferative syndrome based on genotype according to the National Institutes of Health ALPS group

Classification	Abnormality
Type 1a	Mutation on *TNFRSF6* (Fas) gene
Type 1b	Mutation on *TNFSF6* (Fas ligand) gene
Type 2	Mutation on *CASP10* (caspase 10) gene
Type 3	No known mutation

TNFRSF—tumor necrosis factor receptor superfamily

TNFSF—tumor necrosis factor superfamily

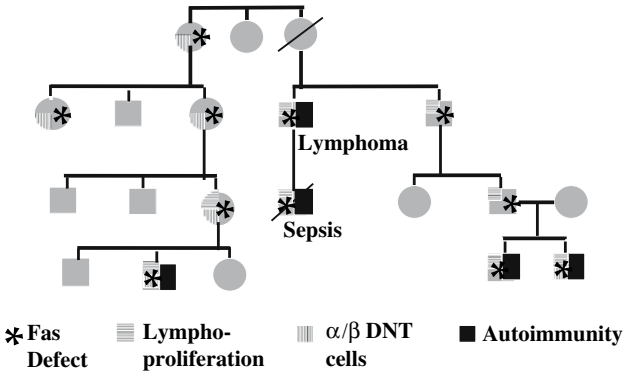

Fig. 3 Expanded pedigree in a family carrying a mutation associated with ALPS type 1a demonstrating the presence of alpha beta DNT-cells, lymphadenopathy, autoimmunity and a mutation in the gene encoding Fas

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 and or environmental factors. This characteristic is similar to the *lpr* and *gld* murine models where strain differences impact on the severity of autoimmunity. The presence of two defective Fas alleles (either homozygous or compound heterozygous) in humans results in a more severe disease typically presenting during the neonatal period and requiring more aggressive therapeutic approaches that has included bone marrow transplantation [14, 15].

A higher than normal frequency of lymphoma became apparent among ALPS type 1a probands and their mutation positive family members [16]. The majority of these cases were B-cell lineage tumors with varying histologic types and formal assessment of the relative risk (RR) for lymphoma yielded a RR for Hodgkin's disease of 51-fold and for non-Hodgkin lymphoma of 14 fold [16, 17]. Tumor tissue from one patient was available and the malignant cells were found to carry the same Fas mutation previously found in genomic DNA. Overall, the lymphomas associated with ALPS have responded to conventional therapy equivalent to malignancies of the same histologic type in non-ALPS patients. One ALPS patient in this series had a history of two different lymphomas, the first successfully treated with chemotherapy while the second presented years later and proved ultimately fatal. Virtually all lymphomas have been found in ALPS type 1a patients with mutations either directly affecting the death domain of Fas or blocking its expression. These defects result in the greatest in vitro defect in Fas mediated lymphocyte apoptosis and had been previously shown to have the highest degree of penetrance in family studies. Thus the magnitude of the apoptotic defect appears to play a role in the risk of developing a lymphoid malignancy.

The ALPS story is approximately 15 years old and has evolved through the successful integration of lessons learned at the "bedside" with work at the "bench." This effort has not only documented the clinical phenotype of ALPS and the underlying molecular mechanisms but also has provided valuable information into 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 with a host of unanswered questions that will only be addressed with continued investigation into patients with ALPS and "ALPS-like" conditions. This story represents

another example of applying the principles that Dr. Robert A. Good pioneered in his extraordinary lifetime drawing upon "experiments of nature" that affect the immune system to further our understanding of immune function in health and disease.

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