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Increased double-negative αβ+ T-cells reveal adult-onset autoimmune lymphoproliferative syndrome in a patient with IgG4-related disease

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Autoimmune lymphoproliferative syndrome (ALPS) is a rare genetic disorder of defective lymphocyte apoptosis characterized by non-malignant expansion of CD4 and CD8 double negative T-cell receptor (TCR) αβ+ T cells (αβ+DNTs) leading to chronic lymphadenopathy, splenomegaly, autoimmune cytopenias and increased susceptibility to malignancy, particularly Hodgkin and non-Hodgkin lymphoma. ALPS is driven by mutations in the FAS-7 fibroblast cell line-associated surface antigen (FAS)/CD95 signaling pathway, with deleterious hemizygous mutations in the FAS gene representing approximately 70% of cases. Other less commonly involved genes are FASL, FADD and CASP10. Affected patients are typically diagnosed in early childhood, however due to incomplete penetrance and variable expressivity, some patients are asymptomatic or may present in adulthood. Genotype-phenotype correlations have also been described, with a more severe disease course associated with dominant negative FAS mutations involving the intracellular death domain, while FAS mutations in the extracellular domain may lead to haploinsufficiency and a milder phenotype.

IgG4-related disease (IgG4-RD) is a systemic immune-mediated fibroinflammatory disease characterized by infiltration of lymphocytes, eosinophils and IgG4-positive plasma cells in various organs with associated fibrosis. Onset of IgG4-RD typically occurs between 50 and 70 years of age and the symptoms vary depending on the affected organ. The most commonly involved organs include the salivary and lacrimal glands, pancreas and biliary tract, kidneys and lymph nodes. Laboratory findings may include elevated serum IgG4, eosinophilia, increased serum IgE and increased serum plasmablasts. Tissue biopsy is the diagnostic gold standard for IgG4-RD, which classically demonstrates a dense lymphoplasmacytic infiltrate, storiform fibrosis, obliterator phlebitis, and an elevation in IgG4+ plasma cells. Various cutoffs for the IgG4/IgG ratio and absolute number of IgG4+ cells per high-power field (hpf) have been proposed, and this metric has been incorporated into the 2019 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria. The co-occurrence of ALPS and IgG4-RD is extremely rare with only two cases currently reported in the English literature. In both prior reports, the patients were initially diagnosed with ALPS with subsequent development of IgG4-RD several years later. Herein we report the first case of an adult patient with IgG4-RD, in which expansion of αβ+DNTs by flow cytometry and subsequent genetic testing ultimately uncovered an underlying diagnosis of ALPS with a pathogenic FAS mutation.

A 63-year-old female with a history of peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS) and invasive ductal carcinoma (IDC) of the breast presented with left axillary lymphadenopathy. At 54 years of age, she was diagnosed with stage IIIA PTCL and treated on a clinical trial with six cycles of cyclophosphamide, etoposide, vincristine, and prednisone alternating with pralatrexate with a complete remission followed by consolidative autologous stem cell transplant. At 59 years of age, she was diagnosed with stage IA (pT1bN0) triple negative IDC of the left breast and treated with lumpectomy, adjuvant carboplatin/paclitaxel and radiotherapy. One-year following treatment for breast cancer, she developed bilateral cervical lymphadenopathy with a waxing and waning course. She was observed for approximately one year until an excisional biopsy of a right cervical lymph node was performed. The biopsy showed no evidence of carcinoma or lymphoma, but rather increased IgG4+ cells (>50/HPF, and >40% IgG4:IgG4 ratio) and multicentric Castleman disease-like features, including interfollicular plasmacytosis, follicles showing multiple germinal centers (“twinning”),
concentric layering of mantle zone lymphocytes around follicles (“onion skinning”) and
germinal center lymphocyte depletion (“regression”) (Figure 1). Serologic examination for HIV
and HHV8 were negative, with the differential diagnosis including IgG4-RD and HHV8-
negative idiopathic multicentric Castleman disease (iMCD). The patient continued to experience
waxing and waning cervical and axillary adenopathy, ultimately leading to a positron emission
tomography (PET) scan, which showed extensive hypermetabolic adenopathy above and below
the diaphragm, along with PET-avid lesions in the pancreas and spleen, concerning for recurrent
lymphoma (Figure 2A). A fine needle aspiration and core biopsy performed on the pancreatic
lesion showed an increase of IgG4+ cells (>25/HPF and an IgG4:IgG ratio > 50%) and storiform
fibrosis (Figure 2B-D). Serum IgG4 and IgE were markedly elevated at 18.33 g/L (normal .11-
1.57 g/L) and 2498 kU/L (normal <100 kU/L), respectively. Peripheral blood B-cell phenotyping
showed an increase in serum CD19+CD38+CD27+ plasmablasts, enumerated at 63.9% of B
cells (normal 0.7%-6%). Overall, the clinical, pathologic, and serologic findings were diagnostic
of IgG4-RD per the 2019 ACR/EULAR classification criteria. Prior to starting treatment for
IgG4-RD, excisional biopsy of a PET-avid right level V cervical lymph node was performed to
rule out recurrent lymphoma. This biopsy showed similar findings of increased IgG4+ cells
(>50/HPF, and >80% IgG4:IgG4 ratio) and multicentric Castleman disease-like features (Figure
3, A-D). Flow cytometry demonstrated an increase in αβ+DNTs (7.1% of lymphocytes and
14.2% of CD3+ T cells), and immunohistochemistry showed paracortical localization of these T-
cells around reactive germinal centers raising suspicion for ALPS (Figure 3, E-H). Peripheral
blood flow cytometry confirmed an increase in circulating αβ+DNTs (4.3% of lymphocytes,
6.1% of CD3+ T cells, 27 cells/uL). Additional laboratory testing revealed elevated soluble FAS
ligand (824 pg/mL) and markedly elevated serum vitamin B12 level above the measured range for
our laboratory instrument (>1000 pg/mL). T-cell receptor beta and gamma chain rearrangements
by next-generation sequencing (NGS) were negative, while targeted NGS mutational analysis
(Stanford Actionable Mutation Panel for Hematopoietic and Lymphoid Malignancies) detected a
pathogenic FAS c.841T>A (p.W281R) mutation, that resulted in an amino acid substitution likely
to be damaging in a region essential for formation of the death-inducing signaling complex
involved in apoptosis induction1. This variant had an allele frequency of 56% suggesting a
germline mutation and confirming the diagnosis of ALPS-FAS1.

Autoimmune lymphoproliferative syndrome caused by FAS mutation (ALPS-FAS) usually
manifests in early childhood at a median age of 2-3 years1. However, due to incomplete
penetrance, variable expressivity, and variation in phenotype according to genotype, a subset of
patients may be asymptomatic or present in adulthood1,2. In these cases acquisition of family and
past medical history of malignancy may be informative, as ALPS-FAS and other germline
diseases are known to predispose to lymphoma and solid tumors. However, in cases of adult-
onset ALPS-FAS, the diagnosis may still be challenging as other hematologic diseases may have
overlapping clinical and pathologic features, including angioimmunoblastic T-cell lymphoma,
Rosai-Dorfman disease, iMCD, and IgG4-RD. Rarely, ALPS may co-occur with one of these
disease entities and as such mask the typical morphologic features of ALPS5,6. While certain
disease working groups, including that for iMCD, currently recommend evaluation for ALPS,
the current consensus diagnostic criteria for IgG4-RD do not7. In our reported case, if flow
cytometry had not been performed to further investigate the increased αβ+DNTs, the diagnosis
of ALPS would likely have been missed as the lymph node histopathological features were
masked by IgG4-RD. Thus, as previously suggested by van de Ven and colleagues6, screening
for increased αβ+DNTs by flow cytometry or ALPS-associated mutations by NGS should be considered in patients with IgG4-RD, particularly in those with other clinical, pathologic, or laboratory features characteristic of ALPS. Identification of ALPS-FAS patients with concurrent IgG4-RD may have significant therapeutic implications, as patients may require chronic therapy or become intolerant to standard immunosuppressive therapy, and thus may benefit from targeted, steroid-sparing therapy such as rituximab or sirolimus.

A mechanistic link between the pathogenesis of ALPS and IgG4-RD is currently unknown. B lymphocytes are involved in the pathogenesis of IgG4-RD as evidenced by marked clinical responses to B-cell-directed therapy with rituximab. It is hypothesized that the oligoclonal expansion of B-cells and plasmablasts leads to IgG4 production, which may contribute to IgG4-RD pathogenesis. Plasmablasts from patients with IgG4-RD show extensive immunoglobulin somatic hypermutation, upregulation of FAS/CD95, and active proliferation and secretion of IgG4. Conceivably, dysregulation of FAS signaling in plasmablasts in ALPS patients may contribute to a preponderance of plasmablasts, and if skewed toward IgG4+ to IgG4-RD. The extensive immunoglobulin somatic hypermutation in plasmablasts suggests a T-cell-dependent germinal center-derived ontology. As such, T lymphocytes have also been implicated in the pathogenesis of IgG4-RD, particularly T follicular helper cells (T<sub>FH</sub>), T follicular regulatory cells (T<sub>regs</sub>), and CD4+ cytotoxic T lymphocytes (CD4 CTLs). Cytokine production by these T-cell subsets appear to contribute to IgG4-RD pathogenesis. Namely, IL-4 and IL-10 produced by T<sub>FH</sub> and T<sub>regs</sub> promote IgG4 isotype switching, whereas IL-1 and TGFβ produced by CD4 CTLs promote fibrosis. While CD4+ T-cells are the best characterized, Carruthers et al has shown that expansion of DNTs may also occur in IgG4-RD, however it is unknown if DNTs contribute to disease pathogenesis. A recent study by Maccari et al. has identified and characterized αβ+DNTs in ALPS patients using RNA sequencing, mass cytometry (CyTOF) and functional cytokine analysis. They find that ALPS-DNTs show a unique surface marker profile with high expression of CD38, CD45RA, CD27, CD28, CLTA4, TIGIT and TIM3, and additionally show up-regulation of IL-10 transcripts and protein levels. Conceivably, ALPS-DNTs may participate in IL-10-mediated class switching of B-cells/plasmablasts to IgG4, however further studies are needed to test this hypothesis.

In conclusion, this case demonstrates the utility of assessing for expanded αβ+DNTs in patients with IgG4-RD, which revealed the diagnosis of ALPS-FAS in our patient. Future studies are needed to investigate a potential mechanistic link between these entities.


Figure Legends

Figure 1. Increased IgG4+ plasma cells in a lymph node with multicentric Castleman disease-like features. Right cervical lymph node excision stained with hemoxylin and eosin (A, 2X objective, 20X total magnification and B, 20X objective, 200X total magnification), IgG4 (C, 20X objective, 200X total magnification) and IgG (D, 20X objective, 200X total magnification).

Figure 2. Radiographic and pathologic features of IgG4-related disease. PET maximum intensity projection image showing hypermetabolic lymphadenopathy above and below the diaphragm along with lesions in the pancreas and spleen (A). Pancreatic core needle biopsy stained with hemxylin and eosin (B), IgG4 (C) and IgG (D). For all micrographs, a 20X objective was used and 200X total magnification is presented.

Figure 3. Expansion of double-negative αβ+ T cells in a background of IgG4-RD. Right level V cervical lymph node excision stained with hemxylin and eosin (A), CD20 (B), IgG4 (C) and IgG (D). Flow cytometry analysis of lymph node gated on CD3+ lymphocytes showing CD4 versus CD8 (E, left) and gated on CD3+CD4-CD8- showing TCRαβ versus TCRγδ (E, right). Immunostaining for CD3 (F), CD4 (G) and CD8 (H). For all micrographs, a 20X objective was used and 200X total magnification is presented.