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Revisiting double-negative T cells in autoimmune lymphoproliferative immunodeficiencies: a case series

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Abstract

Background: Elevated level of double-negative T (DNT) cells is a historical hallmark of autoimmune lymphoproliferative syndrome (ALPS) diagnosis. However, the peripheral blood level of DNT cells might also be compromised in autoimmune lymphoproliferative immunodeficiencies (ALPID) other than ALPS, inattention to which would increase the delay in diagnosis of the underlying genetic defect and hinder disease-specific treatment.

Materials and Methods: This cross-sectional study recruited patients suffering from ALPID (exclusion of ALPS) with established genetic diagnosis. Following thorough history taking, immunophenotyping for lymphocyte subsets was performed using flowcytometry.

Results: Fifteen non-ALPS ALPID patients (60% male and 40% female) at a median (interquartile range: IQR) age of 14.0 (7.6–21.8) years were enrolled. Parental consanguinity and family history of immunodeficiency were present in 8 (53.3%) patients. The median (IQR) age at first presentation, clinical and molecular diagnosis were 18 (4–36) months, 8.0 (4.0–17.0) years, and 9.5 (5.0–20.9) years, respectively. Molecular defects were observed in these genes: *LRBA* (3, 20%), *CTLA-4* (2, 13.3%), *BACH2* (2, 13.3%), *AIRE* (2, 13.3%), and *FOXP3*, *IL2R*ß, *DEF6*, *RASGRP1*, *PIK3CD*, and *PIK3R1* each in one patient (6.7%). The most common manifestations were infections (14, 93.3%), autoimmunity (12, 80%), and lymphoproliferation (10, 66.7%). The median (IQR) count of white blood cells (WBCs) and lymphocytes were 7160 (3690–12,600) and 3266 (2257–5370) cells/mm3, respectively. The median (IQR) absolute counts of CD3+ T lymphocytes and DNTs were 2085 (1487–4222) and 18 (11–36) cells/mm3, respectively. Low lymphocytes and low CD3+ T cells were observed in 3 (20%) patients compared to normal age ranges. Only one patient with *FOXP3* mutation had DNT cells higher than the normal range for age.

Conclusions: Most non-ALPS ALPID patients manifested normal DNT cell count. For a small subgroup of patients with high DNT cells, defects in other IEI genes may explain the phenotype and should be included in the diagnostic genetic panel.

KEYWORDS

ALPID;
ALPS;
flowcytometry;
immune dysregulation;
inborn error of immunity;
primary immune regulatory disorder

Introduction

Inborn errors of immunity (IEIs) refer to a group of inherited defects in the immune system with predominant features of recurrent infections and immune dysregulation, including lymphoproliferation, autoimmunity, atopy, and malignancy.[1]

Some IEI patients display phenotypes similar to autoimmune lymphoproliferative syndrome (ALPS), which was previously referred to as ALPS-like or ALPS-related disorders.[2] Recently, ALPS and ALPS-like disorders have been categorized under the umbrella of "autoimmune lymphoproliferative immunodeficiency (ALPID)".[3] ALPS is a subcluster of ALPID, representing key manifestations of autoimmune cytopenia, non-malignant lymphoproliferation, lymphoma, and a definite mutation in the *FAS*, *FADD,* or *FASLG*.

Other disorders that do not have any abrogation in the FAS signaling but have predominant chronic autoimmune/lymphoproliferative organ involvements (non-ALPS ALPID) are frequently found to have defects in other known genes of IEI. The genes implicated in ALPS-like disorders are dispersed among different categories of IEIs, mainly grouped under diseases of immune dysregulation [regulatory T cell defects (*CTLA-4, LRBA, STAT3 GOF, DEF6, IL2RA*), susceptibility to EBV and lymphoproliferative conditions (*XIAP, MAGT1, RASGRP1, PRKCD, TET2, TNFRSF9, SH2D1A*), familial hemophagocytic lymphohistiocytosis (*UNC13D*), autoimmunity with or without lymphoproliferation (*TPP2, PDCD1*)], but also predominantly antibody deficiency (*PIK3CD* GOF, *PIK3R1* LOF), autoinflammatory disorders (*ADA2, TNFAIP3, PSTPIP1*), (severe) combined immunodeficiency (*STK4, CARD11* GOF, *RELA, ITK, RAG, CD3D*), defects in intrinsic and innate immunity (*IL12RB1, STAT1* GOF), and phenocopies of IEIs (*KRAS, NRAS*).[4–10] Among all, LRBA deficiency, CTLA-4 haploinsufficiency, XIAP deficiency, and STAT3 GOF mutation constitute the majority of cases.[9]

Due to the heterogeneous spectrum of genetic disorders with variable clinical presentations, no specific manifestation can be considered for non-ALPS ALPID. However, it is noteworthy to mention that compared to ALPS, they tend to have higher rates of infections (particularly upper and lower respiratory tract infections), hemophagocytic lymphohistiocytosis (HLH), cutaneous disorders (e.g., alopecia areata), enteropathies (e.g., early-onset inflammatory bowel disease (IBD)), and endocrinopathies (e.g., autoimmune thyroiditis, insulin-dependent diabetes mellitus).[8,9] From an immunologic point of view, elevated levels of double-negative T cells (≥1.5% of total lymphocytes or ≥2.5% of T $CD3^{+11}$ or >6% of T $CD3^{+}TCR\alpha\beta^{+}$ cells[12] have long been one of the major criteria for diagnosing ALPS. DNT cells normally constitute 3–5% of peripheral blood T cells and are deemed to maintain immunological homeostasis.[13] Elevated DNT is not specific for ALPS. Recent studies have shown IEIs other than ALPS, some rheumatologic disorders (such as systemic lupus erythematous (SLE) and ANCA-associated vasculitis), and malignancies (such as lymphomas) may also present high DNT.[14,15] These findings jeopardize the specificity of DNT and downplay its significance in the clinical diagnosis of ALPS patients. In addition, non-ALPS ALPID patients with elevated levels of DNT cells might long be

considered ALPS, which can negatively impact the search for targeted therapy and mislead the diagnosis/treatment (e.g., hematopoietic stem cell transplantation).

The emergence of the ALPID phenomenon has partly helped explain high DNTs in IEIs with abnormalities beyond the Fas-FasL pathway and drawn a border between IEIs and non-IEIs immune dysregulations. In this study, we aimed to evaluate the number of DNTs in non-ALPS ALPID patients to elucidate to what extent DNTs distinguish these categories of disorders and find the rate of abnormal DNTs.

Materials and Methods

Study design and population

This cross-sectional study was designed to enroll fifteen patients with the clinical and genetic diagnosis of primary immune regulatory disorders with phenotypes similar to ALPS (ALPID with the exclusion of ALPS), referred to the immunology clinics of Mofid Children's Hospital. The diagnosis of ALPID was made according to the ALPS diagnostic criteria of the ESID (European Society for Immunodeficiency) registry working party.[12] (Table S1) and ALPID definition provided by a recent study.[3]

The International Union of Immunological Societies (IUIS) expert committee extracted the genes implicated in ALPID disorders from the latest IEI update[4] and a systematic review.[8] The study used a Bayesian Approach to stratify the study population (Tamiji et al., 2019)[30] and was approved by the ethics committee of Shahid Beheshti University of Medical Science, Tehran, Iran (Approval code: IR.SBMU.RETECH.REC.1401.692), according to the guidelines of the Declaration of Helsinki. All patients and, for minorities, their parents signed consent for patients' enrollment in the current study.

Data acquisition

All patients' hospital and outpatient medical records were retrospectively evaluated, and the patients and their parents were directly interviewed for a detailed history taking and evaluation of the current status of the disease. The collected data consisted of (I) demographics data: sex, age at the time of the study, age at onset of symptoms, age at clinical and molecular diagnosis, diagnosis delay, parental consanguinity, family history of immunodeficiency; (II) clinical manifestations including first presentation, infections, autoimmune disorders, atopy, lymphoproliferative disorders, etc. (III) Current treatment and response to treatment. (IV) Genetic study: the result of Whole Exome Sequencing and confirmation of variants by Sanger sequencing.

Immunophenotyping and analysis

For immunophenotyping, the patients' peripheral whole blood was incubated employing a combination of anti-TCRαß- FITC, anti-CD3-APC, anti-CD4-PE, and anti-CD8-PE antibodies (all from BD Biosciences) according to the manufacturer's instruction. After incubation, RBC lysis was performed, the cells were washed twice, and the detection was done using a Sysmex Cyflow Spaceflow cytometer. Analysis of the data was performed via Flowjo 7.6.1 software. A simultaneous complete blood count (CBC) was conducted to determine the absolute number. The results were compared based on the normal range for their ages. DNT cells of equal or more than 2.5% of total T cell counts (≥ 2.5% of T $CD3^+$ cells) were considered high. Statistical analysis was performed using the SPSS software package (SPSS Statistics version 26.0, Chicago, Illinois, USA). For quantitative variables, median and interquartile range (IQR), and for qualitative variables, frequency and percentages were calculated.

Results

Fifteen non-ALPS ALPID patients (9 (60%) male and 6 (40%) female) at a median (IQR) age of 14.0 (7.6–21.8) years were enrolled. Four patients, P4,[16] P8,[17] P9,[18] and P10[ref. 18] are previously reported. Most patients were born to consanguineous parents (far-related (2, 13.3%) or closely related (6, 40%)). Family history of immunodeficiency was present in 8 (53.3%) patients. First presentations of IEI occurred at a median (IQR) age of 18 (4–36) months and comprised of neurologic (4, 26.7%), hematologic (3, 20%), infectious (3, 20%), lymphoproliferative (3, 20%), and autoimmune (2, 13.3%) disorders. Clinical and molecular diagnoses were established in 8.0 (4.0–17.0) years and 9.5 (5.0–20.9) years, respectively. The diagnostic delay was 7.0 (3.0–16.5) years. Molecular defects were observed in the following genes: *LRBA* (3, 20%), *CTLA-4* (2, 13.3%), *BACH2* (2, 13.3%), *AIRE* (2, 13.3%), and *FOXP3*, *IL2RB*, *DEF6*, *RASGRP1*, *PIK3CD*, and *PIK3R1* each in one patient (6.7%).

The most common manifestations were infections in 14 (93.3%) patients [in upper respiratory tract (URT) (8, 53.3%), lower respiratory tract (LRT) (7, 46.7%), oral cavity (5, 33.3%), gastrointestinal tract (3, 20%), skin (1, 6.7%), and in the form of sepsis (1, 6.7%)]. The second most common manifestation was autoimmune disorders in 12 (80%) patients [autoimmune cytopenia (7, 46.7%) including Evans syndrome (3, 20%), isolated autoimmune hemolytic anemia (AIHA) (2, 13.3%), pancytopenia (1, 6.7%), isolated immune thrombocytopenic purpura (ITP) (1, 6.7%)], autoimmune endocrinopathy (5, 33.3%) including insulin-dependent diabetes mellitus (2, 13.3%), autoimmune thyroiditis (4, 26.7%), primary adrenal insufficiency (2, 13.3%), inflammatory bowel disease (IBD) and autoimmune enteropathy (3, 20%), cutaneous disorders (3, 20%)]. Ten patients (66.7%) suffered from non-malignant lymphoproliferations, including splenomegaly (8, 53.3%), lymphadenopathy (7, 46.7%), and hepatomegaly (2, 13.3%). Other less common manifestations included atopic disorders in 4 (26.7%), nephropathies in 3 (20%), and failure to thrive in 2 (13.3%) patients.

The median (IQR) count of white blood cells (WBCs) and lymphocytes were 7160 (3690–12,600) and 3266 (2257–5370) cells/mm3, respectively. The median (IQR) absolute counts of CD3+ T lymphocytes and DNTs were 2085 (1487–4222) and 18 (11–36) cells/mm^3, respectively. Low lymphocytes and low T CD3+ were observed in 3 (20%) patients compared to

normal age ranges. Only one patient with *FOXP3* mutation had an elevated level of DNT cells.

At the time of the study, treatments included intravenous immunoglobulin (IVIG) (8, 53.3%), prophylactic antibiotics (8, 53.3%), steroids (6, 40%), monoclonal antibodies (3, 20%) [rituximab (2, 13.3%), tofacitinib (1, 6.7%)], and sirolimus (1, 6.7%).

Discussion

Flow cytometry plays a nonnegligible role in all historical criteria for the clinical diagnosis of ALPS. With the introduction of next-generation sequencing (NGS), the immunologists' insights on the spectrum of IEIs with phenotypes similar to ALPS experienced a paradigm shift. However, not all clinically-diagnosed ALPS patients undergoing NGS receive a definite molecular diagnosis and there are still gaps in the diagnosis of patients with somatic mutation. In addition, in recent decades, following improvements in diagnosis, management, and overall survival rate of IEIs, chronic autoimmune/autoinflammatory complications had time to develop, and our understanding of the natural history of IEIs has rapidly evolved.[19,20] The introduction of more IEIs with autoimmune/lymphoproliferative features as the predominant phenotype also demands more knowledge of their immunological signature to facilitate genetic interpretation. Therefore, along or prior to NGS, other preliminary and cost-effective methods such as flow cytometry might be considered for early identification of elevated DNT cells, HLA-DR$^+$ T cells, B220$^+$ T cells, CD5$^+$ T cells, CD8$^+$/CD57$^+$ T cells, or decreased regulatory T cells or CD27$^+$ memory B cells.[11,21-24]

In this study, we observed elevated DNT cells in 1 out of 15 (6.7%) non-ALPS ALPID patients. The peripheral expansion of DNT cells has long been a major criterion for diagnosing ALPS. In the current study, we also found normal DNT cells in most non-ALPS ALPID patients, maintaining its specificity at a high level. In a study by Lopez-Nevado.[9] Elevated DNT cells were reported to have an average of 3% (2.4%–3.4%) of CD3+ lymphocytes in ALPS-like patients. Consistently, in our systematic review of ALPS and ALPS-like patients, we previously found an increased DNT cell in 96.3% of ALPS and 75.7% of ALPS-like individuals.[8] Nevertheless, this percentage for ALPS-like patients may be underestimated as this study only included patients who were described in the original literature as having an ALPS-like phenotype (not all patients with defects in ALPS-like genes).

A recent prospective study on ALPID patients found that the clinical and immunological parameters are not helpful in guiding diagnosis for ALPID patients in whom ALPS was excluded. In their cohort of 431 ALPID patients, including 71 patients with ALPS, double-negative T cells had a positive predictive value of 0.7. They suggested considering the CD38+CD45RA+ double negative T cell population as a FAS-controlled subsets.[25]

Therefore, in clinically diagnosed ALPS patients with "negative genetic study," an investigation for genes implicated in other ALPID phenotypes would be suggested. It is unnecessary to reiterate that before such an effort, the biomarker tests with equivocal results should be repeated to ensure the results are not affected by severe lymphopenia or the use of immunosuppressive agents.[3] Evaluation of DNT cells in this study and future similar studies may also introduce other immunologic pathways culminating in the development of DNT cells, providing a framework for future translational research aiming to identify the origin and pathophysiologic roles of DNT cells.

The patient who was found to have increased DNT cells had a *FOXP3* mutation. As summarized in Table 1, the Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) patient was a 14-year-old male suffering from multiple autoimmune/lymphoproliferative complications, including autoimmune enteropathy, insulin-dependent diabetes mellitus, and splenomegaly. Interestingly, the third autoimmune complication, autoimmune thyroiditis, was detected on a routine check-up test right at the time of sampling for this study. This finding along with the previously described tight association between elevated DNT cells and autoimmune disorders,[26] raises the question of whether elevated DNT cells can be used to monitor the deterioration of immune dysregulation. A systematic review of 459 patients with IPEX and IPEX-like syndrome[27] showed patients present autoimmune complications at different time points. The search for biomarkers that can predict the emergence of autoimmunity in IPEX patients is ongoing and recent studies have suggested a correlation between Treg cell-specific demethylated region (TSDR)-demethylated CD4$^+$ T cells and particular cytokines/chemokines with IPEX severity.[28,29]

This study faced several limitations. The participating patients were recruited from a tertiary hospital, and the sample size was too small. The defective genes categorized as non-ALPS ALPID genes were heterogeneous, making the correlation between the phenotype and laboratory findings challenging. In addition, due to the nature of the diseases, a washed-off period before the DNT cell measurement could not be established. As a result, we could not conclude the effect of treatment on the DNT level. For prospective studies, we suggest a greater sample size or separate studies on different genes of ALPID and using novel flow cytometric methods for further classifying DNT cells into their subdivisions.

Statement of Ethics

The study was reviewed and ethical approval was obtained from the Ethics Committee of the Research Center at Shahid Beheshti University of Medical Sciences in accordance with the Declaration of Helsinki (IR.SBMU.RETECH.REC.1401.692). Written informed consent was obtained from participants (or their parent/legal guardian/next of kin) to participate in the study.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

MJ, MM, and ZCH: conceptualization, proposal writing, and obtaining funds for the study; MJ and SNB: data curation,

Table 1 Summary of demographic, clinical, and laboratory findings.

Pt. ID	AOO	AOD (clinical)	AOD (Genetic)	DD	Sex	Age at study	FH	Consanguinity	Initial symptom	Infectious manifestation	Autoimmune manifestations	Atopic manifestations	Lympho-proliferative manifestations	Comments	Current treatment	Mutated gene	WBC (cell/μL)	Lymphocyte (cell/μL)	$CD3^+$ T cell (%)	DNT cell (%)
1	3 y	17 y	20.9 y	17.9 y	M	21.8	Yes	Yes (far-related)	Anemia, Splenomegaly (G6PDD)	Recurrent oral candidiasis, Otitis, recurrent pneumonia, bronchiectasis, Cutaneous infection, severe shingles	Pancytopenia	No	Splenomegaly, para-esophageal lymphadenopathy	Pyoderma gangrenosum, Saddle nose, gingivitis with teeth loss, Perforation of tympanic membrane	Steroid, Co-trimoxazole, Anti-TB	*IL2RB, c.1229C>G, p.P410R*	3500	1050	72.8%	0.90%
2	2 m	3.25 y	6.41 y	6.25 y	M	7.58	No	Yes (far-related)	Pallor and jaundice (AIHA)	Recurrent otitis media	AIHA	No	No	No EBV or HSV infection	Cotrimoxazole, Montelukast, Acyclovir	*RASGRP1, c.934A>G, p.T312A*	7210	2470	71.5%	0.92%
3	1 y	2.5 y	4 y	3 y	F	5.5 y	No	No	Petechia	Recurrent otitis	Evan syndrome, Autoimmune enteropathy	Hyper-reactive airway disease	Lymphadenopathy, Hepatosplenomegaly	GERD, Foot and ankle pain, Sepsis	IVIG, Rituximab, Prednisolone	*LRBA*, c.894G>T, p.K298N	2360	16502	87.6%	1.1%
4	6 m	15 y	18 y	17.5 y	M	19 y	No	No	Febrile seizure (6 and 8 M)	RTI, Pulmonary Aspergillosis, Lung abscess	Vitiligo, Evans syndrome	No	Splenomegaly, Abdominal lymphadenopathies	Interstitial lung disease	Prednisolone, IVIG, ASA, Penicillin V	*LRBA*, c4730-3 T > G	12720	3780	76.2%	0.6%
5	2 m	12 y	12 y	11.83 y	M	12 y	No	Yes (Closely-related)	Pneumonia	Recurrent otitis, pneumonia, chronic rhinosinusitis	No	No	Hepatomegaly	Dilated cardiomyopathy, FTT, Anemia, Bilateral conductive deafness	IVIG, Cotrimoxazole, Spironolactone, Enalapril, Furosemide, L-Carnitine, Ursodeoxycholic	*DEF6*, c.991G>A p.E331K	7500	5370	95.1%	0.2%
6	1 m	6 y	7 y	6.9 y	M	14 y	No	No	IDDM	Chronic diarrhea, Pneumonia, Otitis	Autoimmune enteropathy, IDDM, Autoimmune thyroiditis	No	Splenomegaly	None	IVIG, Cotrimoxazole, Insulin, Levothyroxine	*FOXP3*, c.751_753del, p.Glu251del	12600	8000	79.9%	3.7%
7	1.5 y	2.2 y	2.9 y	1.4 y	F	3 y	No	Yes (Closely-related)	Febrile seizure	COVID-19 infection	Autoimmune thyroiditis	Food allergy to Cow's milk	Cervical lymphadenopathy	Failure to thrive, Urinary reflux with decreased kidney function, Adenoid hypertrophy	Cotrimoxazole, Levebel	*PIK3R1*, c.635-7C>A	13600	5400	67.4%	1%
8	4 y	6 y	14 y	12 y	M	14.25	Yes (Sister died of cytopenia)	No	Lymphadenopathy	Recurrent rhinosinusitis and Otitis	Crohn's disease	Hyper-reactive airway disease, Food allergy	Lymphadenopathy, Splenomegaly	Falsely diagnosed initially with lymphoma	IVIG, Sirolimus, Prednisolone, Mesalamine	*PIK3CD*, c.3061G>A, p.Glu1021Lys	7160	2500	83.4%	1.3%
9	2.5 y	5 y	5 y	2.5 y	F	12 y	Yes (P11: Brother)	Yes (Closely-related)	Photophobia	Dental caries, Oral Candidiasis	PAI	No	No	LSCD and keratoconjunctivitis, Osteoporosis, Nail dystrophy	Hydrocortisone, Fludrocortisone, Calcitriol, Tofacitinib	*AIRE*, p.W78R	3290	1780	71.6%	0.9%
10	2.5 y	9.5 y	9.5 y	7 y	M	16.5 y	Yes (P10: Sister)	Yes (Closely-related)	Photophobia	Dental caries, Oral candidiasis	Alopecia, Vitiligo, PAI, Autoimmune thyroiditis	No	No	keratoconjunctivitis, Nail dystrophy	Hydrocortisone, Fludrocortisone, Calcitriol, Rituximab	*AIRE*, p.W78R	3690	1610	70%	1.0%

(*continues*)

Table 1 Continued.

Pt. ID	AOO	AOD (clinical)	AOD (Genetic)	DD	Sex	Age at study	FH	Consanguinity	Initial symptom	Infectious manifestation	Autoimmune manifestations	Atopic manifestations	Lympho-proliferative manifestations	Comments	Current treatment	Mutated gene	WBC (cell/μL)	Lymphocyte (cell/μL)	$CD3^+$ T cell (%)	DNT cell (%)
11	4 m	8 y	9 y	8.6 y	M	13.5 y	Yes (Mother: P13)	No	Pneumonia	Recurrent diarrhea, Pneumonia, Rhinosinusitis, Otitis, Persistent oral candidiasis, and ulcer	No	No	No	Appendicitis, Adenoid hypertrophy, One episode of Kawasaki-like manifestations	Cotrimoxazole	*BACH2*, p.Tyr729Ter	4900	2499	59.5%	1.5%
12	20 y	35.5 y	36.5 y	16.5 y	F	38 y	Yes (P12: Son)	No	Oral aphthous	Persistent oral candidiasis, Dental caries	No	No	No	No	None	*BACH2*, p.Tyr729Ter	6100	2257	80%	0.5%
13	3 y	20 y	21 y	18 y	M	22 y	Yes (P15: Sister)	Yes (Closely-related)	Hair loss	CMV infection, Chronic diarrhea, Rhinosinusitis	ITP, AIHA, Alopecia, IDDM, Transient hypothyroidism	No	Splenomegaly, Lymphadenopathy	Recurrent nephrolithiasis, Hydronephrosis, Four episodes of hypokalemia, Cholelithiasis	IVIG, Potassium citrate, Insulin	*CTLA-4*, *c.436G>A*, *p.G146R*	7280	3713	43.3%	1.1%
14	22 y	22 y	23 y	1 y	F	24 y	Yes (P14: Brother)	Yes (Closely-related)	Petechia	No	ITP	Eczema	Splenomegaly	No	IVIG, N-plate	*CTLA-4*, *c.436G>A*, *p.G146R*	7100	3266	78.6%	1.3%
15	8 m	4 y	5 y	4.3 y	F	5.25 y	No	No	Lymphadenopathy	Recurrent pneumocystis pneumonia	AIHA	No	Axillary, Cervical, and inguinallymphadenopathy, Splenomegaly	Right hydronephrosis	IVIG, Acyclovir, Cotrimoxazole, Farmentin, Seroflo	*LRBA*, c.52G>A, p.Gly18Arg	16700	5344	79%	2.19%

AOO; age of onset, AOD; age of diagnosis, DD; diagnostic delay, y; year, m; month, FH; family history, WBC; white blood cell, DNT; double-negative T cell, M; male, F; female, TB; tuberculosis, AIHA; autoimmune hemolytic anemia, EBV; Epstein-Barr virus, HSV; herpes simplex virus, CMV; Cytomegalovirus, GERD; gastroesophageal reflux disease, IVIG; intravenous immunoglobulin, RTI; respiratory tract infection, FTT; failure to thrive, IDDM; insulin-dependent diabetes mellitus, LSCD; limbal stem cell deficiency, PAI; primary adrenal insufficiency, ITP; idiopathic thrombocytopenic purpura

investigation, and formal analysis; MM, ZCH, SaS, NE, MK, MV, MS, BSS, HA, KV, GK, SA and SiS: resources, introducing patients; MJ: writing original draft; All authors: writing, review and editing.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Supplementary

Table S1 ESID Diagnostic Criteria for Autoimmune Lymphoproliferative Syndrome

At least one of the following:
- splenomegaly
- lymphadenopathy (>3 nodes, >3 months, non-infectious, non-malignant)
- autoimmune cytopenia (>/= 2 lineages)
- history of lymphoma
- affected family member

And at least one of the following:
- TCRab+CD3+CD4-CD8- of TCRab+CD3+ T cells > 6%
- elevated biomarkers (at least 2 of the following):
  - sFASL > 200pg/ml
  - Vitamin B12 > 1500ng/L
  - IL-10 > 20pg/ml
  - Impaired FAS-mediated apoptosis