Clinical Features and Lymphocyte Immunophenotyping Analysis in Primary Immunodeficiency Patients With Non-transplant Lymphoproliferative Disorders

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Abstract

Purpose: Lymphoproliferative disorders (LPD) comprise a heterogeneous group of diseases and can be classified categorized into the category as being immune dysregulation diseases in primary immunodeficiency diseases (PID) that typically represent as monogenetic X-linked and autoimmune lymphoproliferative diseases (XLP and ALPS). The LPD phenotype extends to other categories in PID and its distribution remains scarce.

Methods: The clinical course and lymphocyte immunophenotyping in PID patients with the LPD phenotype who were referred to the PICAR (Primary Immunodeficiency Care And Research) institute were investigated between 2004 and 2020.

Results: Of the 96 enrolled patients, 31 (32.3%) (median age 144, range 3-252 months) developed the LPD phenotype mainly encompassing lymphadenopathy (in 10 patients), refractory inflammatory bowel disease (IBD)-like with intestinal lymphadenopathy in 8 and hepatosplenomegaly (in 7) during long-term follow-up (median 144, range 3-252 months). The LPD phenotype was most frequently present in the categories of antibody deficiency (in 7: 2 CVID, 2 TTC37, PIK3CD, PIK3R1 and AICDA each), phagocyte (in 6: 4 CYBB, 1 STAT1 and 1 IFNRG1), immune dysregulation (in 6: 2 FOXP3, 2 XIAP and 2 HLH), combined immunodeficiencies (in 5: 2 IL2RG, 1 CD40L, 1 ZAP70 and 1 unknown) and syndromic features (in 4: 2 STAT3-LOF, 1 WAS and 1 ATM) as well as three patients with anti-IFN- γ autoantibodies. Those with the LPD phenotype had a significantly higher mortality rate than those without ($p=0.0016$) despite a similar age at onset (median 66 vs 44 months) and follow-up duration (138 vs 144 months). EBV virus load was only detectable in the PIK3CD and HLH patient each. An increased senescent (CD8+CD57+) and CD21-low components, and disturbed transitional B (CD38+IgM++), plasmablast B (CD38++IgM-), memory B (CD19+CD27+) and T_{EMRA} (CD27-IgD-) components were often observed in cross-sectional immunophenotyping. However, except patients with CD40L and ZAP70 mutations, those with profound T cell defects who bear almost naïve (T and B cells) components and extreme low in the remaining subsets components trended to develop their LPD phenotype.

Conclusion: The spectrum of the LPD phenotype in one third of the PID patients overriding the categorical boundaries had higher mortality and heterogeneous lymphocyte disturbances, especially higher senescent and CD21-low but lower memory B subpopulations. Large-scale longitudinal studies of PID patients are needed to validate the correlation between immunophenotype and the LPD status.

Introduction

Lymphoproliferative disorders (LPD) comprise a heterogeneous group of diseases characterized by the uncontrolled production of lymphocytes that mainly cause monoclonal lymphocytosis, lymphadenopathy and bone marrow infiltration [1, 2]. Patients with genetic mutations responsible for primary immunodeficiency diseases (PID) or inborn errors of immunity (IEI) can present with “combination” phenotypes of refractory allergy, recurrent infections, autoimmune, autoinflammation and malignant transformation [3]. These latter three disorders mainly underlie immune imbalance and dysregulation with the potential to increase susceptibility to the development of the LPD phenotype.

In the update PID/IEI classification, patients classified into the “immune dysregulation” category nomenclaturally bear the LPD phenotype [4]. Among them, X-linked lymphoproliferative disease 1 (XLP1) patients with *SH2D1A* (SH2 domain 1A) or *SAP* (SLAM-associated protein) genetic mutations have been shown to be predisposed to acquire chronic and persistent EBV infection behaving as natural killer/T cell LPD overlapping recurrent infectious mononucleosis, hemophagocytic lymphohistiocytosis (HLH) and chronic active EBV infection (CAEBV), and to be at risk of the further development of lymphoma [5]. Resembling XLP1 patients, the XLP2 phenotype caused by *XIAP* (inhibitor of apoptosis, X-linked) mutations is recognized by huge splenomegaly and refractory inflammatory bowel disease (IBD)-like diarrhea [6]. In addition, mutations in the genes encoding Fas or associated proteins cause autoimmune lymphoproliferative syndrome (ALPS) that cannot adequately modulate apoptosis to prevent autoinflammation [7]. Beyond the “immune dysregulation” category encompassing the so-called typical LPD disorders, those patients with common variable immunodeficiency (CVID), immunodeficiency, polyendocrinopathy, enteropathy and X-linked (IPEX) or IPEX-like, severe combined immunodeficiency (SCID or CID), Wiskott-Aldrich syndrome (WAS), ataxia-telangiectasia (ATM) or Chediak-Higashi syndrome could have the potential to develop the LPD phenotype, especially in accompanying with EBV infection [8, 9].

To overview the LPD phenotype based on the update European Society for Immunodeficiencies (ESID) [10], we defined the LPD phenotype as encompassing hepatosplenomegaly, lymphadenopathy, pulmonary granuloma, intestinal lymphadenopathy, hemophagocytic lymphohistiocytosis (HLH), and lymphoma transformation. The LPD phenotype may orchestrate or interact with an imbalance in T regulatory cells (Treg), Th17 cells, T follicular help cells and others [11]. In this study, we assessed the distribution of the LPD phenotype in a cohort of patients using the updated categories and investigated whether lymphocyte disturbance may be associated with this phenotype and whether it can affect the prognosis.

Material And Methods

Patients

Since 2003, 1220 individuals (838 suspected cases and 382 related persons) have been referred to the Primary Immunodeficiency Care and Research (PICAR) Institute for molecular/genetic diagnosis of PID. Two hundred and eighty patients were identified as having PID (Supplemental Fig. 1) and were classified according to the 2019 updated PID categories [4], as in our previous study [15]. The patients and healthy controls provided informed and written consent for data collection and publication of this study. All human samples were obtained under protocols approved by the Institutional Review Board at Chang Gung Memorial Hospital (protocol 202001665A3, 202002403A3, and 201902037A3) and met the Institutional Review Board standards for ethical conduct of research with human subjects. Written informed consent was obtained from all individual participants included in the study. Basic immunologic functions were assessed according to clinical characteristics and candidate genes were sequenced from complement DNA synthesized from RNA and confirmed again

using genomic DNA as previously described. [12–14] For those with the LPD phenotype [7, 8, 10], we investigated their clinical features, lymphocyte imbalance and categorical distribution.

Assessment of memory, T follicular helper, senescent, Th17, Treg, transitional and plasmablast B cells

Peripheral blood mononuclear cells (PBMCs) were processed using Ficoll (GE Healthcare, Marlborough, MA, USA) to form a single cell suspension, which was then stained with the following monoclonal antibodies against cell surface and intracellular antigens of memory T, Treg, T follicular helper, memory B, and CD21-low B cells as in our previous study [12, 16]: anti-CD4-PE (clone SK3), CD4 FITC (clone SK3), CD8-PE (clone SK1), CD19-PerCP-Cy5.5 (clone HIB19), CD25-FITC (clone 2A3), CD21-FITC (clone B-ly4), IgD-PE (clone IA6-2), CD27-APC (clone M-/t271), CD45RO-PE (clone UCHL1), CD154-PE (clone TRAP1), CCR7-APC (CD197, clone 3D12), CXCR5 PerCP-Cy5.5 (clone RF8B2), CD38-PE (clone HIT2), IgM-FITC (clone FA-DA4), CD57-PE (clone TBO1), IgD-PE (clone IA6-2) (all from BD Pharmingen and eBioscience, San Diego, CA).

Statistical analysis

The phenotypes, treatment, and prognosis of our patients with and without LPD were compared. The first follow-up day was defined as the age at onset, and the last follow-up day was the near recent clinical visit. The survival analysis, t-test and chi-square were performed using GraphPad Prism software, and a p value of <0.05 was considered to indicate a statistically significant difference.

Results

The Distribution Of Lpd In The Update Categories

Among 280 recognized patients at our PICAR institute, 96 had long-term follow-up (mean 130, median 144, range, 3-252 months) in our institute. The autoimmune-related disorders in our cohort [15] included severe atopic dermatitis, herpes-like dermatitis, idiopathic thrombocytic purpura, alopecia, Takayasu’s vasculitis, albinism and the LPD phenotype. A total of 31 patients had the LPD-related phenotype, including lymphadenopathy (in 10 patients), hepatosplenomegaly (in 7), refractory diarrhea (IBD-like with intestinal lymphadenopathy in 7), granuloma (lung and skin in 3), hemophagocytic lymphohistiocytosis (HLH in 3), lymphoma (in 2), and splenomegaly (in 1) (Table 1). The LPD phenotype was most present in the antibody deficiency (7 of 19 patients), phagocyte (6 of 19), and immune dysregulation (6 of 6) categories.

Table 1
The lymphoproliferative disorder (LPD) phenotypes in the PID patients classified by every category

Symptoms/Signs	Total	Predominantly B	Combined	Phenocopies	Phagocyte	Syndromic	Immune Dys.
Subgroup patient number	72+24#	19	9	4	19	15	6
Lymphadenopathy	10	2	2	3	3		
Refractory diarrhea (IBD-like)	8	3	1			1	3
Hepatosplenomegaly	7	1	5		1		
Hemophagocytic lymphohistiocytosis	3						3
Lymphoma	2					2	
Granuloma (subgroup)	3						
Lung	(2)				2		
Skin	(1)					1	
Splenomegaly	2	1			1		
Liver cirrhosis	1	1					
Arthropathy	1	1					
#These 24 patients classified into the four categories of defects in innate immunity (9 patients), autoinflammation (3 patients), complement (1 patient) and unclassified (11 patients) diseases were free of lymphoproliferative disorders at the time of the study.							

The diagnosis of ALPS was excluded by normal TCRα-β subsets, absent autoimmune pancytopenia and all wild-type genetic analysis in the Fas or associated proteins, confirmed by whole exome sequencing.

The most common genetic defect was CYBB (4 patients), followed by TTC37, IL2RG, STAT3-LOF, XIAP and FOXP3 in two patients each, and PIK3CD, PIK3R1, CD40L, ZAP70, STAT1, IFNRG1, WAS and ATM in one patient each (Table 2). The genetic defects of three patients with anti-IFN-γ autoantibodies, two patients with CVID, two with HLH, and one with CID could not be identified.

Table 2
Lymphoproliferative disorders and opportunistic infections in non-transplant PID patients

Patient / sex	Tested age (Months)	Mutations or/ clinical diagnosis	Clinical presentation					
			Opportunistic infection	Autoimmune/Inflammation disorder				Basic Treatment [mortality]
				Lymphadenopathy	Hepatosplenomegaly	IBD-like diarrhea	Others	
Predominantly antibody deficiencies (19)								
P52 / M	273	Undefined/CVID			+			[IVIG, prednisolone]
P71 / F	744	Undefined/CVID		+				IVIG
P429 / F	5	TTC37, 3724 ins A, Tyr1169Stop				+	Liver cirrhosis	IVIG, prednisolone
P321* / M	NB	TTC37, homozygous DelK1155H, Fs*2;				+		[IVIG, prednisolone]
P622 / F	11	PIK3CD, c.3061G>A; p. E.1021K	EBV, CMV			+		IVIG, prednisolone, mTOR, HSCT
P728 / M	208	PIK3R1, c.1425+2T>G		+			Arthropathy	IVIG, prednisolone, mTOR, anti-TNF, anti-IL6
P323 / F	20	AICDA, Del 37Asp38Ser					Splenomegaly	IVIG
Combined T and B immunodeficiencies (9)								
P9 / M	10	IL2RG, Trp74Gly	Oral candidiasis, PJP		+		erythroderma	IVIG, HSCT
P394 / M	3	IL2RG, c.854G>A, skip exon 6	Oral candidiasis, PJP		+			[IVIG]
P325 /M	1	Undefined/CID	BCG, candidiasis	+	+			IVIG, HSCT
P72 / M	121	CD40L, Del 347A	Oral candidiasis,	+	+			IVIG
P635 / M	22	ZAP70. c.1561 G>A; Asp 521 Asn	Oral candidiasis, PJP		+	+	hepatitis	IVIG, prednisolone, HSCT
Phenocopies of PID (Auto IFN-γ Abs) (4)								
P32 / F	613	Undefined/recurrent NTM	NTM, varicella, Talaromyces (Penicillium) marneffei	+				Anti-NTM, prednisolone
P188 / F	624	Undefined/recurrent NTM	NTM	+				Anti-NTM
P197 / M	675	Undefined/recurrent NTM	NTM	+				Anti-NTM, prednisolone
Congenital defects of phagocyte number, function or both (19)								
P54 / M	66	STAT1, Thr385Met	BCG, candidiasis	+	+		Hypothyroidism	IVIG, prednisolone, HSCT

Abbreviations: CVID: common variable immunodeficiency; IBD: inflammatory bowel disease; HLH: hemophagocytic lympho-histiocytosis; NTM: non-tuberculosis mycobacterial infections; WAS: Wiskott-Aldrich syndrome; ATM: ataxia-telangiectasis; There were no lymphoproliferation syndrome in the categories of Autoinflammatory disorders and defects in innate immunity

Patient / sex	Tested age (Months)	Mutations or/ clinical diagnosis	Clinical presentation				
			Opportunistic infection	Autoimmune/Inflammation disorder			
				Lymphadenopathy	Hepatosplenomegaly	IBD-like diarrhea	Others
							Basic Treatment [mortality]
P22 / M	31	Gp91, Del 1693G	BCG, candidiasis				Splenomegaly [IVIG, prednisolone, HSCT]
P389 / M	4	Gp91, c. Del846, 847 TG	BCG, aspergillosis				Lung granuloma prednisolone, HSCT
P404 / M	3	Gp91, Del exon 1, 2	BCG				Lung granuloma [prednisolone, HSCT]
P450 / M	14	Gp91, c.1028C>A, p.Thr343Lys	BCG, aspergillosis	+			Prednisolone, HSCT
P132 / M	87	IFNGR, 818Del 4 nu.	BCG	+			Anti-NTM, IFN-
Combined immunodeficiencies with associated or syndromic features (12)							
P225 / M	158	WAS, Int(+5)G>A, skip exon 8					Severe atopic dermatitis, lymphoma [IVIG, prednisolone, chemotherapy for DLBCL]
P162 / M	157	SATA3, Gln469Arg					Cutaneous granuloma IVIG, prednisolone
P315 / M	134	STAT3, IVS10(-2)A>G, skip exon 11				+	IVIG
P178 / M	120	ATM, Arg805Stop, Lys486Fs					CNS lymphoma [Chemotherapy for lymphoma]
Diseases of immune dysregulation (9)							
P296 / M	12	XIAP, Int 5 (+1) G>A	Candidiasis			+	HLH-like [IVIG, Prednisolone, anti-TNF, TPOG-CHOP]
P682 / M	NB	XIAP c.997-1001 del" CAGAA" 5 nu; DelQ333 Fs15stop					[IVIG, Prednisolone, anti-TNF, TPOG-CHOP]
P405 / M	2	FOXP3, Met370Leu	Candidiasis			+	[IVIG, mTOR, anti-TNF, prednisolone]
P407 / M	2	FOXP3, c.736-2A>C	Candidiasis			+	Erythroderma, [IVIG, mTOR, anti-TNF, prednisolone]
P182 / M	217	Undefined/HLH	EBV				HLH TPOG 2004HS
P214 / F	144	Undefined/HLH					HLH TPOG 2004HS
Abbreviations: CVID: common variable immunodeficiency; IBD: inflammatory bowel disease; HLH: hemophagocytic lympho-histiocytosis; NTM: non-tuberculosis mycobacterial infections; WAS: Wiskott-Aldrich syndrome; ATM: ataxia-telangiectasis; There were no lymphoproliferation syndrome in the categories of Autoinflammatory disorders and defects in innate immunity							

Clinical Features Beyond The Lpd Phenotype

As expected, patients with combined T and B cell deficiency (caused by IL2RG, ZAP70 and CD40L mutations), defective phagocyte reactive oxygen species (caused by CYBB mutations) and IFN- γ /IL12/23 dysfunction (due to autoantibodies to IFN- γ and STAT1 mutations) often suffered from opportunistic infections, such as mucosa candidiasis, nontuberculosis mycobacteria infections, aspergillosis, varicella and Talaromyces (Penicillium) marneffeii. Only two patients with PIK3CD (P622) and an unknown genetic defect (P182) had a detectable EBV or/and CMV viral load in all 31 patients. Two patients with WAS and ATM did not have detectable viral load, however they both developed lymphoma.

Compared to the PID patients without the LPD phenotype, those with the LPD phenotype had a significantly higher mortality rate ($p=0.0061$, log-rank test; $p=0.0124$, Wilcoxon test; Fig. 1A) during a similar follow-up period (median 138 vs 144 months, range 4-252 months vs 3-212 months, $p=0.3470$; Fig. 1B) even though there was no significance in the age at recognition (median 66, range 0.003 to 675 months; vs median 40, range 1 to 972 months, $p=0.3672$; Fig. 1C). The age at onset of the LPD phenotype was not significantly different between the survivors and the mortalities (median 81, range 1 to 675 months; vs median

5, range 0.03 to 273 months, $p=0.1730$; Fig. 1D), although the survivors tended to have an older age at onset of the LPD phenotype. Among the PID patients with the LPD phenotype, the prognostic factors for survival were not correlated to opportunistic infections, hepatosplenomegaly, and IBD-like diarrhea (chi-square test; Supplemental Table 1). Notably, the presence of lymphadenopathy seemed to be an independent survival factor, because the 10 patients with lymphadenopathy all survived.

All patients had bronchiectasis except for three who received early effective IVIG (P 323) hematopoietic stem cell transplantation (HSCT) (P450) and prophylactics (P134). The treatments affecting nature courses of the LPD phenotype mainly included regular IVIG (in 21), immunosuppressants (prednisolone in 19 patients), mTOR inhibitors (rapamycin in 2), biologics (in 2), chemotherapy based on the TPOG (Taiwan Pediatric Oncologic Group) guideline (in 4), and HSCT (in 9) if their courses were refractory and suitable donors were available to rescue them from the LPD phenotype, including IBD-like diarrhea, erythroderma, autoimmune hepatitis and lung granuloma.

Lymphocyte disturbance in the PID patients with the LPD phenotype

Among the PID patients with the LPD phenotype, immunophenotyping in the “predominantly antibody deficiencies” category showed decreased memory B but higher CD4+ effector memory T and CD21-low B cell components (in Table 3), and especially higher Tfh and senescent components in the patients with *PIK3CD* and *PI3K3R1* mutations (as shown in P728 in Fig. 2). All patients in the “combined T and B immunodeficiency” category had fewer memory cells. Extremely low numbers of T cells with an almost naïve pattern were common in three patients with IL2RG (P9 and P394) and undefined (P325) mutations, but not in two patients with respective CD40L and ZAP70 mutations (P72 and P635) whose T (CD4+ and CD8+) and B (CD19+) cell components were normal and senescent cell components were higher.

Table 3
Lymphocyte subpopulation percentages in the PID patients with the lymphoprolifera

Patient / sex	Tested age (Months)	Mutations or/ clinical diagnosis	B CD19										CD21-low	Transit	Plas
			Naive		Switch		Non-S		ExM						
Naive		TCM		TEM	TEMRA	Tfh	Naive	TCM	TEM	TEMRA		CD57			
Predominantly antibody deficiencies															
P52 / M	273	Undefined/CVID	24.7												
			92.0			0.5			6.7	0.7	3.2	0.4	0.2		
P71 / F	294	Undefined/CVID	3.7												
			86.1			0.0			13.9	0.0	10.7	12.7	3.1		
P429 / F	5	TTC37, 3724 ins A, Tyr1169Stop	4.6												
			91.6			0.0			2.1	6.3	18.4	13.2	0.2		
P321* / M	NB	TTC37, homozygous DelK1155H, Fs*2;	5.2												
			90.7			0.1			1.9	7.3	20.2	14.3	0.1		
P622 / F	11	PIK3CD, c.3061G>A; p. E.1021K	14.9												
			83.6			0.1			0.5	15.8	66.9	0.1	0.1		
P728 / M	208	PIK3R1, c.1425+2T>G	1.2												
			70.9			0.0			3.5	22.6	87.8	0.0	0.0		
P323 / F	20	AICDA, Del 37Asp38Ser	7.2												
			85.6			1.0			9.2	4.2	22.1	0.6	0.1		
Combined T and B immunodeficiencies															
P9 / M	10	IL2RG, Trp74Gly	95.4												
			90.2			0.2			0.1	3.1	0.2	0.1	0.0		
P394 / M	3	IL2RG, n.854G>A, skip exon 6	97.9												
			90.4			0.1			0.2	2.2	0.1	0.0	0.2		
P325 /M	1	Undefined/CID	90.8												
			89.0			0.2			1.2	2.2	0.7	2.0	1.2		
			10.2												
P72 / M	121	CD40L, Del 347A	82.0				0.2			1.2	16.6	22.4	0.2	0.4	
P 635 / M	22	ZAP70,. c.1561 G>A;	18.3												
		p. Asp 521 Asn	32.3				9.4			7.0	51.3	3.2	73.3	1.3	
Phenocopies of PID (Auto IFN-γ Abs)															
			3.6												
P32 / F	613	Undefined/recurrent NTM	84.6				8.2			2.2	5.1	28.2	10.8	13.9	
			1.9												
P188 / F	624	Undefined/recurrent NTM	90.5				4.8			2.1	2.6	4.7	31.8	15.4	
			7.9												

Pink background indicates higher than the normal range; Gray background indicates lower than the normal range, mainly based on our age-matched controls

[^] Kverneland AH, Streitz M, Geissler E, Hutchinson J, Vogt K, Boës D, Niemann N, Pedersen AE, Schlickeiser S, Sawitzki B. Age and gender leucocytes variance Jun;89(6):543-64. doi: 10.1002/cyto.a.22855. Epub 2016 May 3. PMID: 27144459.

^{^^} Garcia-Prat M, Álvarez-Sierra D, Aguiló-Cucurull A, Salgado-Perandrés S, Briongos-Sebastian S, Franco-Jarava C, Martin-Nalda A, Colobran R, Montserrat I, reference values in a healthy pediatric population. Cytometry B Clin Cytom. 2019 May;96(3):223-233. doi: 10.1002/cyto.b.21728. Epub 2018 Oct 17. PMID: 30

Patient / sex	Tested age (Months)	Mutations or/ clinical diagnosis	B CD19							
			Naive		Switch	Non-S	ExM	CD21-low	Transit	Plasma
P197 / M	675	Undefined/recurrent NTM	79.3		12.3	6.2	2.2	8.2	6.8	2.3
Congenital defects of phagocyte number, function or both										
		t	8.4							
P54 / M	66	STAT1, Thr385Met	70.1		12.1	10.2	7.6	16.2	4.2	8.6
P22 / M	31	Gp91, Del 1693G	10.2							
			74.8		9.8	7.8	7.4	18.2	3.8	1.6
P389 / M	4	Gp91, n. Del846, 847 TG	12.2							
			75.8		9.9	9.7	4.6	20.1	5.4	4.2
P404 / M	3	Gp91, Del exon 1, 2	20.2							
			85.2		7.4	2.5	4.9	17.4	3.9	3.2
P450 / M	14	Gp91, n.1028C>A, p.Thr343Lys	11.4							
			26.6		13.6	31.6	28.2	19.9	4.9	2.4
P132 / M	87	IFNGR, 818Del 4 nu.	9.7							
			52.4		12.8	10.4	24.4	14.8	5.6	4.1
Combined immunodeficiencies with associated or syndromic features										
P225 / M	158	WAS, Int(+5)G>A, skip exon 8	1.7							
			60.8	5.4		1.5		32.2	20.5	6.5
P162 / M	157	STAT3, Gln469Arg	10.2							
			81.3	8.0		1.9		8.8	8.6	5.3
		STAT3, IVS10(-2)A>G, skip exon 11	24							
P315 / M	134		95.2	1.3		1.4		2.1	23.1	11.8
			2.2							
P178 / M	120	ATM, Arg805Stop, Lys486Fs	59.5	7.6		30.0	2.9	12.6	2.1	1.0
Diseases of immune dysregulation										
P296 / M	12	XIAP, Int 5 (+1) G>A	8.2							
			78.6	4.2	3.6		13.6	30.2	11.4	23.7
P682 / M	NB	XIAP c.997-1001 del"CAGAA" 5 nu; DelQ333 Fs15stop	10.2							
			74.2	3.2	2.6		20.0	25.9	8.9	20.2
			24.8							
P405 / M	2	FOXP3, Met370Leu	32.8	8.4	4.8		54.0	7.2	4.2	10.2
			20.2							
P407 / M	2	FOXP3, c.736-2A>C	28.8	5.8	2.2		63.2	6.8	10.2	22.2
			10.2							

Pink background indicates higher than the normal range; Gray background indicates lower than the normal range, mainly based on our age-matched controls

^A Kverneland AH, Streitz M, Geissler E, Hutchinson J, Vogt K, Boës D, Niemann N, Pedersen AE, Schlickeiser S, Sawitzki B. Age and gender leucocytes variance Jun;89(6):543-64. doi: 10.1002/cyto.a.22855. Epub 2016 May 3. PMID: 27144459.

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Patient / sex	Tested age (Months)	Mutations or/ clinical diagnosis	B CD19							
			Naive		Switch	Non-S	ExM	CD21-low	Transit	Plas
P182 / M	117	Undefined/HLH	45.2	21.3	17.8	15.7		18.4	4.2	2.1
			14.3							
P214 / F	144	Undefined/HLH	60.4	18.4	14.5	6.7		13.2	4.5	1.9
Pink background indicates higher than the normal range; Gray background indicates lower than the normal range, mainly based on our age-matched controls										
^ Kverneland AH, Streitz M, Geissler E, Hutchinson J, Vogt K, Boës D, Niemann N, Pedersen AE, Schlickeiser S, Sawitzki B. Age and gender leucocytes variance Jun;89(6):543-64. doi: 10.1002/cyto.a.22855. Epub 2016 May 3. PMID: 27144459.										
^^ Garcia-Prat M, Álvarez-Sierra D, Aguiló-Cucurull A, Salgado-Perandrés S, Briongos-Sebastian S, Franco-Jarava C, Martin-Nalda A, Colobran R, Montserrat I, reference values in a healthy pediatric population. Cytometry B Clin Cytom. 2019 May;96(3):223-233. doi: 10.1002/cyto.b.21728. Epub 2018 Oct 17. PMID: 30										

The patients with autoantibodies to IFN- γ had higher components of transitional B, plasmablast B, effector CD4 memory, T_{EMRA} and senescent cells (as shown in P188 in Fig. 3). Their Th17 component was elevated to 8% (in the second lane of Fig. 3), which was the highest in our cohort. For phagocyte disorders, the patients with X-linked granulomatous diseases (XL-CGD) had higher memory B and senescent components (representative demonstration of P450 in Fig. 4). Their Th1-polarizing Tfh components (in the third lane of Fig. 4) could elicit a robust IFN- γ response to possibly induce a cytokine storm, consequently driving the HLH crisis.

Our syndromic PID patients with WAS, hyper IgE syndrome and ATM had decreased memory (B and T) but still higher senescent components. Two XIAP patients had higher CD21-low and transitional B components (Fig. 5A). Two IPEX patients had higher exhausted memory (IgD-IgM-, double negative cell) components (Fig. 5B).

Overall, the number of dysregulated lymphocyte subpopulations (total 22 recorded subpopulations in Table 3) was not significantly correlated with mortality. In extreme cases, a patient (P349) with 19 abnormal subpopulations and another (P229) with all normal subpopulations both died.

Conclusions

Approximate one third (31 of 96) of the PID patients presented with the LPD phenotype and higher mortality rate when compared to those without the LPD phenotype. Eleven fatal PID patients with the LPD phenotype may have been ascribed to the absence of timely HSCT, except for two with TTC37 and ATM mutations (P429 and P178), neither of whose respective liver cirrhosis and brain lymphoma benefitted from HSCT. Those patients with XIAP and FOXP3 mutations should early receive HSCT to reconstruct multiple immunophenotype disturbances [27] that theoretically contribute to recurrent HLH and refractory IBD-like diarrhea. Such lymphocyte disturbances of higher components of CD21-low B, transitional B, exhausted B and senescent T cells were not only demonstrated in the category of “immune dysregulation” as XIAP, FOXP and HLH patients, but also expanding into other categories in which the LPD phenotype could also result from an imbalance in Tfh and memory components.

In immunophenotyping analysis, higher senescent components (CD8+CD57+) with low IL-2 but pronounced IFN- γ production [17, 18] were the most common. The senescent cells are telomere-dependent and low number in children but accumulate with age as seen with the TEMRA cells in patients who had anti-IFN γ autoantibodies (P32, P188 and P157) and the patient with a *PIK3CD* mutation who persisted EBV and CMV infections (P622) presenting with hyper IgM syndrome [12, 19] and PASLI (P1106-Activating mutation causing Senescent T cells, Lymphadenopathy, and Immunodeficiency) disease [20]. Higher percentages of senescent cells were noted in two patients (58.4% in the patient with WAS and 20.9% in the patient with ATM) who developed lymphoma in the absence of detectable EBV virus load. Whether a higher CD8+CD57+T component increases the potential for malignant transformation in PID patients with the LPD phenotype remains to be elucidated.

Through CD40L, ICOS and IL-21 signaling [21, 22], circulating Tfh cells can induce B cells to class switch and differentiate into memory and plasmablast (CD38++IgM-) cells as long-lived humoral immunity [23]. Lower Tfh cell components in those with B cell deficiency negatively affected the process of memory and plasmablast cells and consequently converged to hypogammaglobulinemia in the “predominantly antibody deficiencies” category. In contrast, the patients with *PIK3CD* and *PIK3R1* mutations maintained higher circulating Tfh components, which could reflect that the higher number of Tfh cells in their huge hepatosplenomegaly and intestinal lymphadenopathy *in situ*, continuously reacted with chronic EBV or CMV infections leading to refractory IBD-like diarrhea.

In the patients with WASP, STAT3, XIAP and anti-IFN γ autoantibodies, the higher transitional B component (CD38++IgM++) surviving through negative/positive selection, as well as more plasmablast (CD38++IgM-) cells, mutually produced more auto-antibodies to target intestines and blood cells presenting as the LPD phenotype of IBD-like diarrhea, lymphadenopathy and HLH.

Furthermore, CD21-low B cell components belonging to CD21+CD27+ memory B cells were undetectable in cord blood and increased in autoimmune diseases [24], as all LPD patients except those with profound T cell defects (the CID patients) who simultaneously had diminished CD21-low B cell development because of defective support from T cell pool. In particular, the XL-CGD patient (P450) was like untreated rheumatoid arthritis patients with higher exhausted memory B cells (ExM, double negative IgM-IgD-) [25], in whom a cytokine storm was overwhelmingly active and therefore could induce HLH-like macrophage activation syndrome. The other four patients (P132, P405, P407, P450) in two categories of phagocyte disorders and immune dysregulation

had also higher ExM memory B cells and increased the risk of HLH. Thus, based on such observation, further studies are warranted to investigate whether higher ExM B cell components relate to cytokine storms and therefore progress into HLH status.

Consistent with our previous study that Treg and Th17 cells remain at a lower range in most PID patients [15], relatively higher Treg populations in patients with *PIK3R1*, *ZAP70*, *IFNGR* and *STAT3* mutations may compensate an overactive inflammation/autoimmune response during LPD status. In other words, the highest Th17 component in the patients with anti-IFN γ autoantibodies inferred that Th17 augmentation positively enhanced the higher levels of anti-IFN γ autoantibodies. As well as anti-CD20 deleting B-cell therapy (rituximab) to eradicate anti-IFN γ autoantibodies as far as possible [26], anti-Th17 antagonists (secukinumab) may be an additional treatment to attenuate Th17 signaling for decreasing anti-IFN γ autoantibodies.

This study should be interpreted in a light of its limitations. First, the cross-sectional assessments of lymphocyte immunophenotypes at diagnosis of the LPD phenotype may have been, at least partially, confounded by accompanying subclinical infections. Longitudinal and large-scale follow-up comparisons of lymphocyte immunophenotypes in patients with and without LPD should more accurately reflect the whole phenotypic spectra. Second, in addition to quantitative evaluations, functional studies of cytokine profiles (IL-10, TGF- β , and IL17), Treg contact suppression and immunoglobulin class-switch under Tfh co-culture will hopefully be assessed in future studies if more blood samples become available in pediatric-onset PID patients. Third, the CID patients with profound T cell defects had lower components of all immunophenotypes except naïve lymphocytes, which hindered clarifying the relationship between the LPD phenotype and extreme lymphocyte subpopulations including, at least, CD21-low, transitional, plasmablast B cells, Tfh, TEMRA and senescent cells. Fourth, as well as relative lymphocyte components, absolute counts might indicate the occurrence and development of the LPD phenotype if we could have obtained these two values at the same time.

In conclusion, increased senescent CD8+CD57+ and CD21-low components and disturbed transitional B, plasmablast B and TEMRA components were observed in our 31 Taiwanese PID patients with the LPD phenotype who had higher mortality. EBV infection was only detected in two (2/31), but unrelated to lymphoma transformation in the other two. However, in those with profound T cell defects rather than the CD40L and ZAP70 mutations, whether the predominantly naïve lymphocyte (T and B cell) components and extremely low in the remaining populations were correlated with their LPD phenotype remains to be determined.

Declarations

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Consent to participate: Written informed consent was obtained from all individual participants included in the study.

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Figures

Figure 1

Figure 1

The PID patients with the LPD phenotype had a significantly higher mortality rate ($p=0.0061$, log-rank test; $p=0.0124$, Wilcoxon test) compared to those without the LPD phenotype (A) during a similar follow-up period (median 138 vs 144 months $p=0.3470$) (B) and age at recognition (median 66 vs 40 months, $p=0.3672$) (C). However, the age at onset of the LPD phenotype was not significantly different (median 81 vs median 5 months, $p=0.1730$) in those who survived and died (D).

Figure 2

Healthy Control

Patient (P728)

Figure 2

In immunophenotyping analysis, patient P728 with a PIK3R1 mutation had decreased memory B (switched IgD+CD27+ and non-switched IgD-CD27+memory CD19 cells; 3.5% vs 53.5% in the second lane) but higher CD4+ effector memory T (CD45RO+CCR7- CD4 cells, 83.6% vs 21.3% in the second lane) and CD21-low B (87.8% vs 12.9% in the first lane) cell components, especially higher Tfh (45.9% vs 17.6% in the third lane) and senescent CD8+CD57+ components (57.4% vs 32.1% in the first lane) compared with the healthy control (B).

Figure 3

Figure 3

Patient P188 with autoantibodies to IFN- γ had higher components of transitional B (CD38++IgM++ 31.8% vs 3.9% in the second lane), plasmablast B (CD38++IgM- 15.1% vs 7.2% in the first lane), effector CD4 memory (CD45RO+CCR7- CD4 cells; 67.8% vs 52.5% in the third lane), TEMRA (CD45RO-CCR7- CD4 cells; 8.5% vs 3.3% in the third lane), senescent CD8+CD57+ (43.7% vs 32.8% in the third lane) and the highest Th17 components (8.8% vs 2.0% in the second lane) compared to the healthy control.

Figure 4

Figure 4

The X-linked granulomatous diseases (XL-CGD) patient P450 had higher non-class-switched memory B (IgD+CD27+memory 31.6% vs 13.6% in the first lane) and senescent CD8+CD57+ components (32.4% vs 13.1% in the second lane). However, the normal Tfh components (CD4+CXCR5+; 30.8% vs 23.9% in the third lane) were skewed to Th1 (CXCR3+CCR6-; 56.9% vs 24.7% in the third lane) and could elicit a robust IFN- γ response to possibly induce a cytokine storm consequently driving the HLH crisis.

Figure 5

Figure 5

The XIAP patient (P682) had higher CD21-low (25.9% vs 11.9%) and transitional B components (8.9% vs 4.4%) under CD19 gating (A). The IPEX patient (P407) had higher exhausted memory (IgD-CD27- or IgD-IgM-; 63.2% vs 8.3%) double negative cell components under CD19 gating (B).

Supplementary Files

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