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Clinical features and lymphocyte immunophenotyping analysis in primary immunodeficiency patients with non-transplant lymphoproliferative disorders

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ABSTRACT

Lymphoproliferative disorders (LPD) comprise a heterogeneous group and are originally classified into the "Disease of immune dysregulation" category. Of 96 Taiwanese patients during 2003–2022, 31 (median 66, range 0.03–675 months) developed LPD, mainly including palpable lymphadenopathy (in 10 patients), intestinal lymphadenopathy associated with refractory inflammatory bowel disease (IBD in 8) and hepatosplenomegaly (in 7) during long-term follow-up (median 144, range 3–252 months). They distributed in the categories of antibody deficiency (2 CVID, 2 *TTC37*, *PIK3CD*, *PIK3R1* and *AICDA* each), phagocyte (4 *CYBB*, 1 *STAT1* and 1 *IFNRG1*), immune dysregulation (2 *FOXP3*, 2 *XIAP* and 2 HLH), combined immunodeficiencies (2 *IL2RG*; *CD40L*, *ZAP70* and unknown each), syndromic features (2 *STAT3*-LOF, 1 *WAS* and 1 *ATM*) and three with anti-IFN-γ autoantibodies. An increased senescent (CD8 + CD57+) and CD21-low, 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 and trended to develop LPD.

1. Introduction

Lymphoproliferative disorders (LPD) comprise a heterogeneous group of diseases characterized by the uncontrolled production of lymphocytes that manifest as monoclonal lymphocytosis, lymphadenopathy and bone marrow infiltration [1,2]. Patients harboring genetic mutations responsible for primary immunodeficiency diseases (PID) or inborn errors of immunity (IEI) can exhibit composite phenotypes of refractory allergy, recurrent infections, autoimmune, autoinflammation and malignant transformation [3] that all underlie immune imbalance and dysregulation prone to the development of the LPD phenotype.

In the updated PID/IEI classification, patients classified into the "disease of immune dysregulation" category nomenclaturally bear the LPD phenotype [4], originally encompassing monogenetic X-linked and autoimmune lymphoproliferative diseases (XLP and ALPS). X-linked lymphoproliferative disease (XLP1 and XLP2) patients with respective *SH2D1A* (SH2 domain 1A or *SAP*, SLAM-associated protein) and *XIAP* (inhibitor of apoptosis, X-linked) genetic mutations predispose to persistent EBV infection that drive them to natural killer/T cell LPD overlapping recurrent infectious mononucleosis, hemophagocytic lymphohistiocytosis (HLH) and huge splenomegaly and refractory inflammatory bowel disease (IBD)-like diarrhea especially in XLP2; and to lymphoma transformation in both XLP1 and XLP2 patients [5,6]. Furthermore, defective apoptotic signaling by mutations in the genes encoding Fas or associated proteins in ALPS patients cannot adequately carry out apoptosis to prevent autoinflammation [7]. Beyond typical LPD disorders of XLP and ALPS in the "disease of immune dysregulation" category, those patients with common variable immunodeficiency

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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 patent) and unclassified (11 patients) diseases were free of lymphoproliferative disorders at the time of the study.

(CVID), severe combined immunodeficiency (SCID or CID), Wiskott-Aldrich syndrome (WAS), ataxia-telangiectasia (ATM) or Chediak–Higashi syndrome have the potential to develop the LPD phenotype, while often accompanying EBV infection [8,9].

To overview the LPD phenotype based on the updated European Society for Immunodeficiencies (ESID) 10], we defined the LPD phenotype to mainly encompass palpable lymphadenopathy (cutaneous, some related to HLH), intestinal lymphadenopathy (related to IBD), hepatosplenomegaly, and lymphoma transformation. The LPD phenotype could arise or parallel with an imbalance in T follicular help, C21-low B, Th17, and T regulatory (Treg) cells [11]. In this study, we assessed the distribution of the LPD phenotype in a cohort of patients based on the updated PID categories and investigated whether lymphocyte disturbance might associate with this phenotype and correlate to the prognosis.

2. Material and methods

2.1. Patients

During 2003–2022, 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 and further management. Two hundred and eighty patients were identified as having PID (Supplemental Fig. 1) and were classified according to the 2022 updated PID categories [4], as in our previous study [12]. The patients and healthy controls provided informed and written consent for the 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 (protocols 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 [13–15]. For those with the LPD phenotype [7,8,10], we assessed their clinical features, lymphocyte subsets and categorical distribution.

2.2. Assessment of lymphocyte immunophenotyping

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 T follicular helper, CD21-low B, Th17, Treg and memory 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), CD21-FITC (clone B-ly4), IgD-PE (clone IA6–2), CD27-APC (clone M−/t271), CD45RO-PE (clone UCHL1), CCR7-APC (CD197, clone 3D12), CXCR5 PerCP-Cy5.5 (clone RF8B2), CD38-PE (colon HIT2), IgM-FITA (clone FA-DA4), CD57-PE (colon TBO1), IgD-PE (clone IA6–2), IL17A-PE (clone N49–653) and FOXP3 (clon 259D/7C) (all from BD Pharmingen and eBioscience, San Diego, CA). The subsequent gating flowchart was shown in the supplementable Fig. 2. To minimize the tested volumes and simplize cross-reaction for interpretation, we separated eight tubes form peripheral whole blood and isolated mononuclear cells for staining and analysis (in supplementable Table 1).

2.3. Statistical analysis

The phenotypes, treatment, and prognosis of our patients with LPD were reviewed. The first follow-up day was defined as the age at onset, and the last follow-up day was the most 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.

3. Results

3.1. The distribution of LPD in the update categories

Ninety-six PID patients among 280 recognized patients had long-term follow-up (mean 130, median 144, range, 3–252 months) in our PICAR institute. The autoimmune/autoinflammation-related (immune dysfunction or dysregulation) 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 that included palpable lymphadenopathy (in 10 patients), hepatosplenomegaly (in 7), intestinal lymphadenopathy associated with refractory IBD-like diarrhea (in 7), granuloma (lung and skin in 3), HLH-related lymphadenopathy (in 3), lymphoma (in 2), and splenomegaly (in 1) (Table 1). The LPD phenotype was most common observed in the “antibody deficiency” (7 of 19 patients), followed by the “phagocyte” (6 of 19), and the “immune dysregulation” category (6 of 6) categories.

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). For three patients with anti-IFN-γ autoantibodies, two with common variable immunodeficiency (CVID), two with hemophagocytic lymphadenopathy (HLH), and one with combined immunodeficiency (CID), genetic defects have not been identified to date.

We ruled out the diagnosis of ALPS in our patients by demonstrating normal double-negative CD3+ CD4-CD8-TCRαβ+ subsets, the absence of autoimmune pancytopenia, and wild-type genetic analysis in the Fas or associated proteins. This exclusion was further verified through

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/Autoinflammation in LPS disorder				
				Lymphadenopathy*	Hepatosplenomegaly	IBD-like diarrhea	Others	Basic Treatment [mortality]
Predominantly antibody deficiencies (19)								
P52 / M	273	Undefined/CVID			+			[IVIG, prednisolone]
P71 / F	744	Undefined/CVID		+				IVIG
P429 / F	5	TTC37, heterozygous c.3426dupA, p. Ala1143 fs*3; c. T3507G, p. Tyr1169Stop				+	Nodular regenerative hypertrophy, liver cirrhosis	IVIG, prednisolone
P298 / M	NB	TTC37, homozygous c.2464-5delAA, p. Lys1155 fs*2				+		[IVIG, prednisolone]
P622 / F	11	PIK3CD, heterozygous c. G3061A, p. Glu1021Lys	EBV, CMV			+		IVIG, prednisolone, mTOR, HSCT
P728 / M	208	PIK3R1, heterozygous c.T1425 + 2G, p. Asp434Glu fs*249 (skip exon9)		+			Arthropathy	IVIG, prednisolone, mTOR, anti-TNF, anti-IL6
P323 / F	20	AICDA, homozygous c.110-5delGTGCAC, Del 37Asp38Ser					Splenomegaly	IVIG
Combined T and B immunodeficiencies (9)								
P9 / M	10	IL2RG, c.T220G, p. Trp74Gly	Oral candidiasis, PJP		+		erythroderma	IVIG, HSCT
P394 / M	3	IL2RG, c.G854A, p. Glu253Gly fs*9 (skip exon6)	Oral candidiasis, PJP		+			[IVIG]
P325 /M	1	Undefined/CID	BCG, candidiasis	+	+			IVIG, HSCT
P72 / M	121	CD40LG, c.326del A, p.Asn109Thr fs*19	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, heterozygous c.C1154T, p. Thr385Met	BCG, candidiasis	+	+		Hypothyroidism	IVIG, prednisolone, HSCT
P22 / M	31	Gp91, c.1679delG, p. Gly560Glu fs*17	BCG, candidiasis				Splenomegaly	[IVIG, prednisolone, HSCT]
P389 / M	4	Gp91, c.846-7delTG, p.Cys282Stop	BCG, aspergillosis				Lung granuloma	prednisolone, HSCT
P404 / M	3	Gp91, c.1-141del (lose exon 1–2)	BCG				Lung granuloma	[prednisolone, HSCT]
P450 / M	14	Gp91, c.C1028A, p. Thr343Lys	BCG, aspergillosis	+				Prednisolone, HSCT
P132 / M	87	IFNGR1, c.818delTTAA, p. Asn274His fs*1	BCG	+				Anti-NTM, IFN-
Combined immunodeficiencies with associated or syndromic features (12)								
P225 / M	158	WAS, c.G734 + 5A, p. Pro188Met fs*14 (skip exon 7)					Severe atopic dermatitis, lymphoma	[IVIG, prednisolone,

(*continued on next page*)

Table 2 (*continued*)

Patient / sex	Tested age (Months)	Mutations or/ clinical diagnosis	Clinical presentation					
			Opportunistic infection	Autoimmune/Autoinflammation in LPS disorder				
				Lymphadenopathy*	Hepatosplenomegaly	IBD-like diarrhea	Others	Basic Treatment [mortality]
								chemotherapy for DLBCL]
P162 / M	157	STAT3, c.A1046G, p. Gln469Arg					Cutaneous granuloma	IVIG, prednisolone
P315 / M	134	STAT3, c.A1050-2G, p.Leu351Asp fs*400 (skip exon 11)				+		IVIG
P178 / M	120	ATM, c.C2413T, p. Arg805Stop; Del c.1402–3 AA (exon 9) Lys 468 Glu fs*17					CNS lymphoma	[Chemotherapy for lymphoma]
Diseases of immune dysregulation (9)								
P296 / M	12	XIAP, c.G1099 + 1 A, p.Val353Met fs*25 (skip exon 5)	Candidiasis			+	HLH-like	[IVIG, Prednisolone, anti-TNF, TPOG-CHOP]
P682 / M	NB	XIAP, c.997–1001 CAGAA del., p. Gln333Gly fs*15						[IVIG, Prednisolone, anti-TNF, TPOG-CHOP]
P405 / M	2	FOXP3, c.A1108C, p. Met370Leu	Candidiasis			+		[IVIG, mTOR, anti-TNF, prednisolone]
P407 / M	2	FOXP3, c.A736–2C, p. Leu246Ala fs*159 (skip exon 8)	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 Auto-inflammatory disorders and defects in innate immunity.

* Underlying infectious pathogens causing lymphadenopathy had been excluded.

whole exome sequencing.

3.2. Clinical features beyond the LPD phenotype

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 signal impairment (due to autoantibodies to IFN-γ and *STAT1* mutations) often suffered from opportunistic infections of mucosal candidiasis, nontuberculosis mycobacteria infections, aspergillosis, varicella and *Talaromyces (Penicillium) marneffei* (in Table 2). Detectable EBV or/and CMV viral loads were explored in only two patients (one patient P622 with the *PIK3CD* mutation and the other P182 with an unknown genetic defect) in all 31 patients. However, none EBV virus load was detected in two patients with *WAS* (P225) and *ATM* (P178) mutations developing 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 the 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 with intestinal lymphadenopathy (chi-square test; Supplemental Table 2). Notably, the presence of palpable lymphadenopathy seemed to be an independent survival factor, because the 10 patients with palpable lymphadenopathy all survived.

All patients, with the exception of three who underwent early effective intravenous immunoglobulin (IVIG) (P 323), hematopoietic stem cell transplantation (HSCT) (P450), and prophylactic antibiotics (P134), presented with bronchiectasis. The natural progression of the LPD phenotype was positively influenced by various effective treatments, including regular IVIG (in 21), immunosuppressants (prednisolone in 19 patients), mTOR inhibitors (rapamycin in 2), biologics (in 2), chemotherapy based on the Taiwan Pediatric Oncologic Group (TPOG) guideline (in 4), and HSCT (in 9) when suitable donors were available to rescue their refractory status of intestinal lymphadenopathy associated with IBD-like diarrhea, erythroderma and autoimmune hepatitis (P635) and lung granuloma (P389).

3.3. Lymphocyte disturbance in PID patients with the LPD phenotype

In comparison to the healthy controls, PID patients with the LPD phenotype within "predominantly antibody deficiencies" category showed decreased memory B but higher CD4+ effector memory T and CD21-low B cell components (in supplemental Table 3). Notably, patients with *PIK3CD* and *PI3K3R1* mutations had higher Tfh and senescent components as illustrated in P728 in Fig. 2. Within the "combined T and B immunodeficiency" category, all patients had reduced memory cell components. Three patients with *IL2RG* mutations (P9 and P394) and an undefined mutation (P325) were expected as markedly low T cell numbers. In contrast, two patients with *CD40L* and *ZAP70* mutations (P72 and P635) had normal T (CD4+ and CD8+) and B (CD19+) cell components, and elevated senescent cell components.

Patients with autoantibodies to IFN-γ had increased levels of

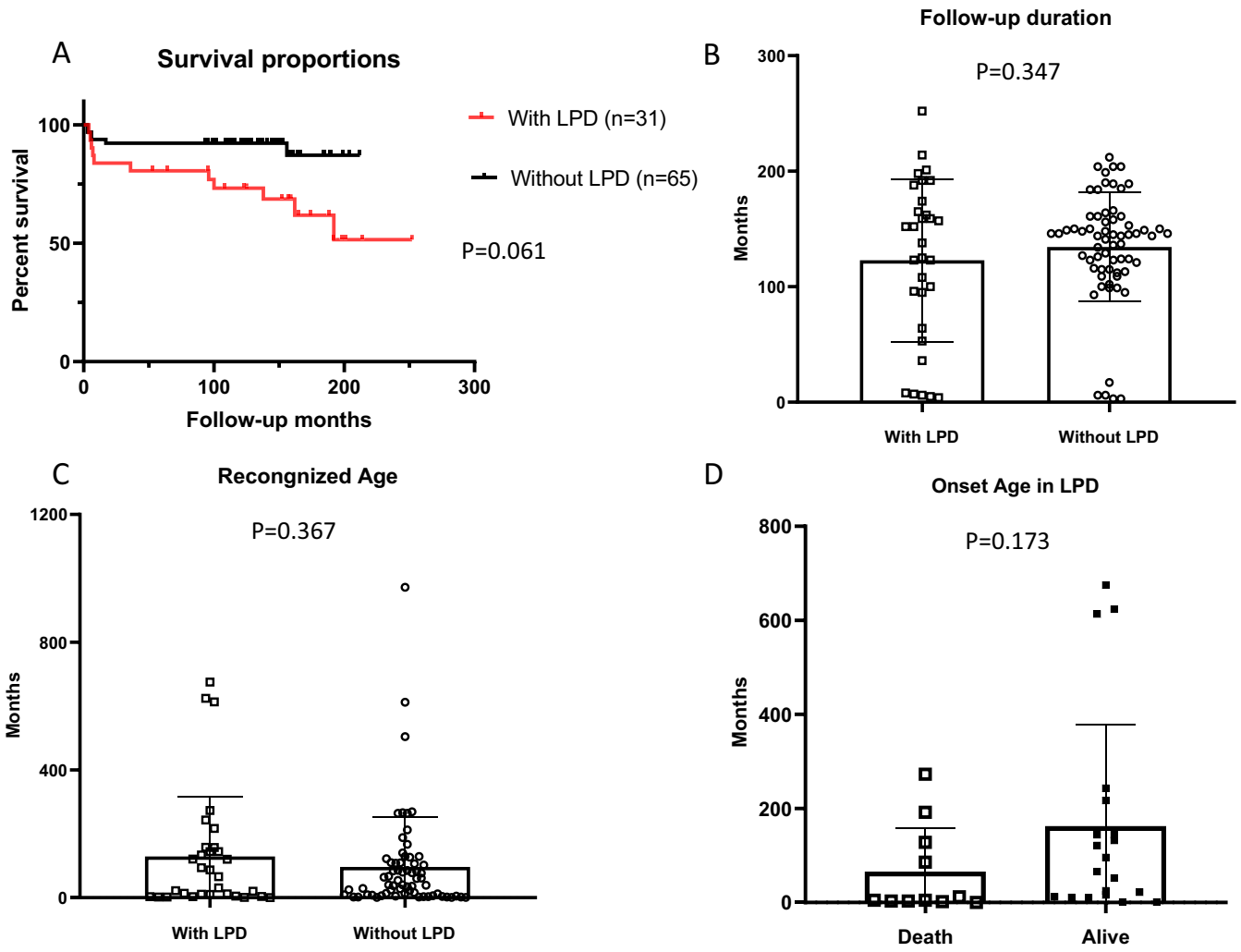

Fig. 1. The PID patients with the LPD phenotype had a significantly higher mortality rate ($p = 0.0061$, log-rank test; $p = 0.0124$, Wilcoxon test) compared to those without the LPD phenotype (A) during a similar follow-up period (median 138 vs 144 months $p = 0.3470$) (B) and age at recognition (median 66 vs 40 months, $p = 0.3672$) (C). However, the age at onset of the LPD phenotype was not significantly different (median 81 vs 5 months, $p = 0.1730$) in those who survived and died (D).

transitional B cells, plasmablast B cells, effector CD4 memory cells, terminally differentiated effector memory CD4 cells (T_{EMRA}), and senescent cells, as exemplified in P188 in Fig. 3. Notably, their Th17 component was elevated to 8% (shown in the second lane of Fig. 3), representing the highest value in our cohort.

In the case of phagocyte disorders, specifically in X-linked granulomatous diseases (XL-CGD) patients, there was a higher presence of memory B cells and senescent components, as demonstrated in the representative display of P450 in Fig. 4. Additionally, their T follicular helper (Tfh) components with Th1-polarizing characteristics (seen in the third lane of Fig. 4) could potentially trigger a robust IFN-γ response, possibly leading to a cytokine storm and driving the development of macrophage activation syndrome (MAS) or hemophagocytic lymphadenopathy (HLH)-like crisis.

In our syndromic PID patients with WAS, hyper IgE syndrome and ATM, there was a notable decrease in memory (both B and T), yet higher senescent components persisted. Two XIAP patients had elevated levels of CD21-low and transitional B components, as depicted in Fig. 5A. Two IPEX patients showed heightened exhausted memory (IgD-IgM-, double-negative cell or ExM) components, as illustrated in Fig. 5B.

The overall mortality did not significantly correlate with the number of abnormal lymphocyte subpopulations, encompassing a total of 22 recorded items as outlined in Supplemental Table 3. Notably, in extreme cases, a patient (P349) with 19 dysregulated subpopulations and another (P22) with all normal subpopulations both experienced mortality.

4. Discussions

Approximate one third (31 of 96) of the PID patients presented with the LPD phenotype. In their lymphocyte disturbances, higher components of CD21-low B, transitional B, exhausted B and senescent T cells revealed not only in the category of “disease of immune dysregulation” among XLP2, IPEX and HLH patients, but also additional imbalances in Tfh and memory components that all expanded into other PID categories. Except for the unfortunate cases of liver cirrhosis in *TTC37* patients and brain lymphoma in ATM patients, both of which showed no improvement with HSCT, the nine fatal PID patients with the LPD phenotype underline the critical importance of timely HSCT. It is noteworthy that those XLP2 and IPEX patients necessitate early HSCT intervention. This is pivotal for the reconstruction of multiple immunophenotype disturbances [17], aiming to effectively address recurrent HLH and refractory IBD-like diarrhea. Timely HSCT not only becomes a therapeutic imperative but also holds the potential to avert severe consequences in patients grappling with these challenging conditions.

Next, we elucidated the lymphocyte immunophenotyping disturbance in our PID patients with the LPD phenotype. The most common immunophenotyping disturbance was higher senescent components

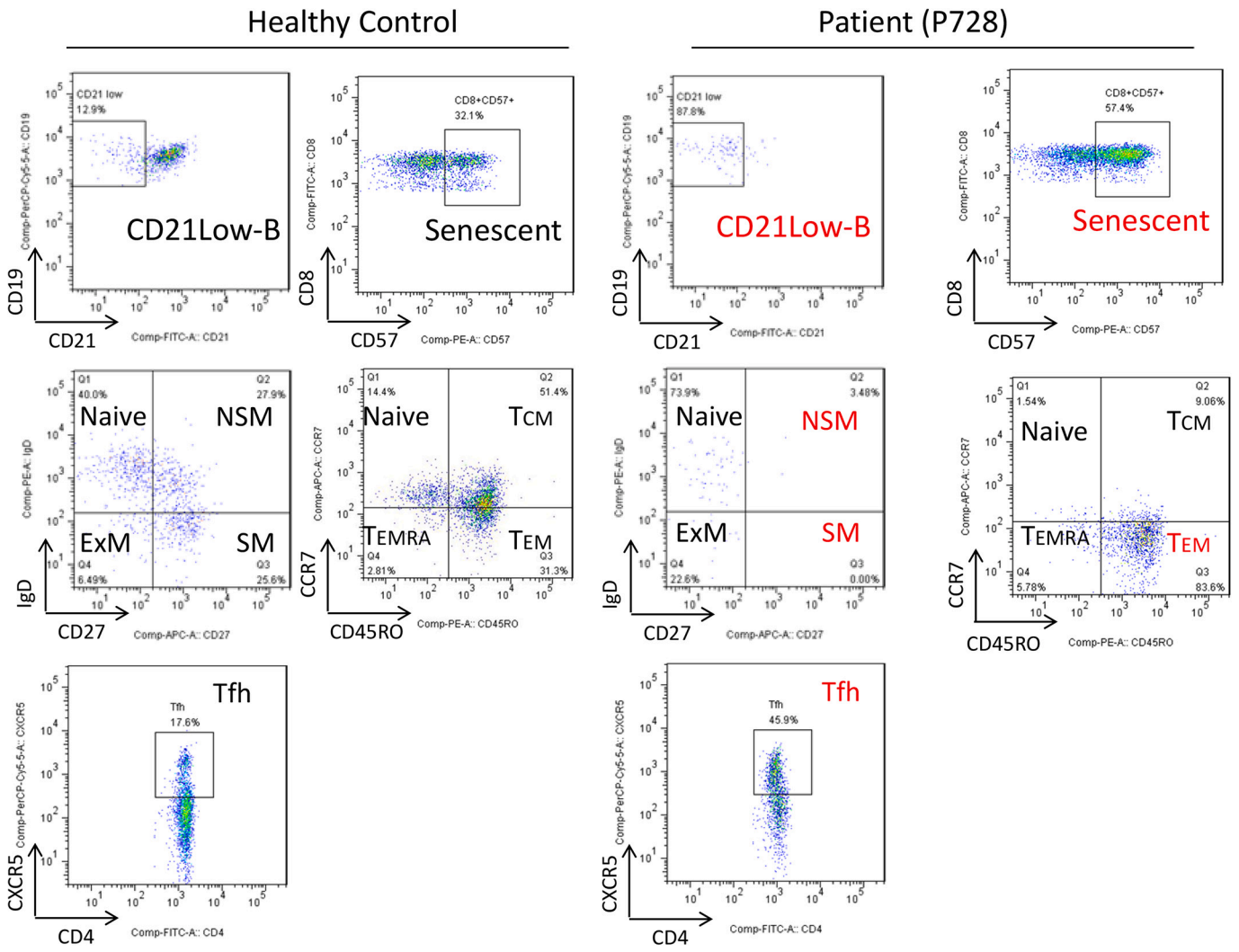

Fig. 2. In immunophenotyping analysis, patient P728 with a *PIK3R1* mutation had decreased memory B (switched IgD + CD27+ and non-switched IgD-CD27 + memory CD19 cells; 3.5% (0.00% plus 3.48%) vs. 53.5% (25.6% plus 27.9%) in the second lane) but higher CD4+ effector memory T (CD45RO + CCR7- CD4 cells, 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).

(CD8 + CD57+) with pronounced IFN-γ production [18]. The senescent cells are telomere-dependent and low number in children but accumulate with age as seen with the TEMRA cells in patients with anti-IFNγ autoantibodies (P32, P188 and P157) and one with a *PIK3CD* mutation (P622) who persisted with EBV and CMV infections presenting as the hyper IgM syndrome [12] and PASLI (P110δ-Activating mutation causing Senescent T cells, Lymphadenopathy, and Immunodeficiency) disease [19,20]. Higher percentages of senescent cells were also found in two WAS and ATM patients (58.4% and 20.9%) who developed lymphoma without a detectable EBV virus load. Whether an increased CD8 + CD57+ T cell component signifies premature aging and amplifies the risk of malignant transformation in PID patients with the LPD phenotype warrants further exploration.

Interestingly, argumentative status existed in the level of Tfh components [21]. Tfh cells can enhance B cells to class switch and differentiate into memory and plasmablast (CD38++IgM-) cells for long-lived humoral immunity [22]. The process of memory and plasmablast cells was severely impacted by lower Tfh cell components in patients with B cell shortage, ultimately led to hypogammaglobulinemia. In contrast, the patients with *PIK3CD* and *PIK3R1* mutations whose hypogammaglobulinemia was in need of regular immunoglobulin infusion maintained higher circulating Tfh components, reflecting in situ more Tfh located within their huge hepatosplenomegaly and intestinal lymphadenopathy due to continuous activation from chronic EBV and CMV infections or gut inflammation.

Moreover, antibody-enhancing B cell pools for autoimmune disorders maintain higher transitional B component (CD38++IgM++), CD21–low B cell and plasmablast (CD38++IgM-) [22,23] in agreement with our patients with WASP, STAT3 and XIAP mutations and anti-IFNγ autoantibodies who could present with lymphoma, intestinal lymphadenopathy associated with IBD diarrhea and HLH-related lymphadenopathy. However, those with profound T-cell defects (the CID patients) and presenting as the LPD phenotype had a diminished antibody-enhancing B cell pool because of insufficient enhancement from the T cell pool [23].

In particular, the XL-CGD patient (P450) was like untreated rheumatoid arthritis patients with higher exhausted memory B cells (ExM, double negative IgM-IgD-) [24], in whom a cytokine storm was overwhelmingly active and therefore could induce HLH-like macrophage activation syndrome. However, his HLH did not occur by a successful HSCT. Higher ExM memory B cells in the other three patients (P132, P405, P407; 1 *IFNRG* and 2 *FOXP3* mutations) spread two categories of "phagocyte disorders" and "disease of immune dysregulation" that could have higher ExM B cell components and infer premature exhaustion due

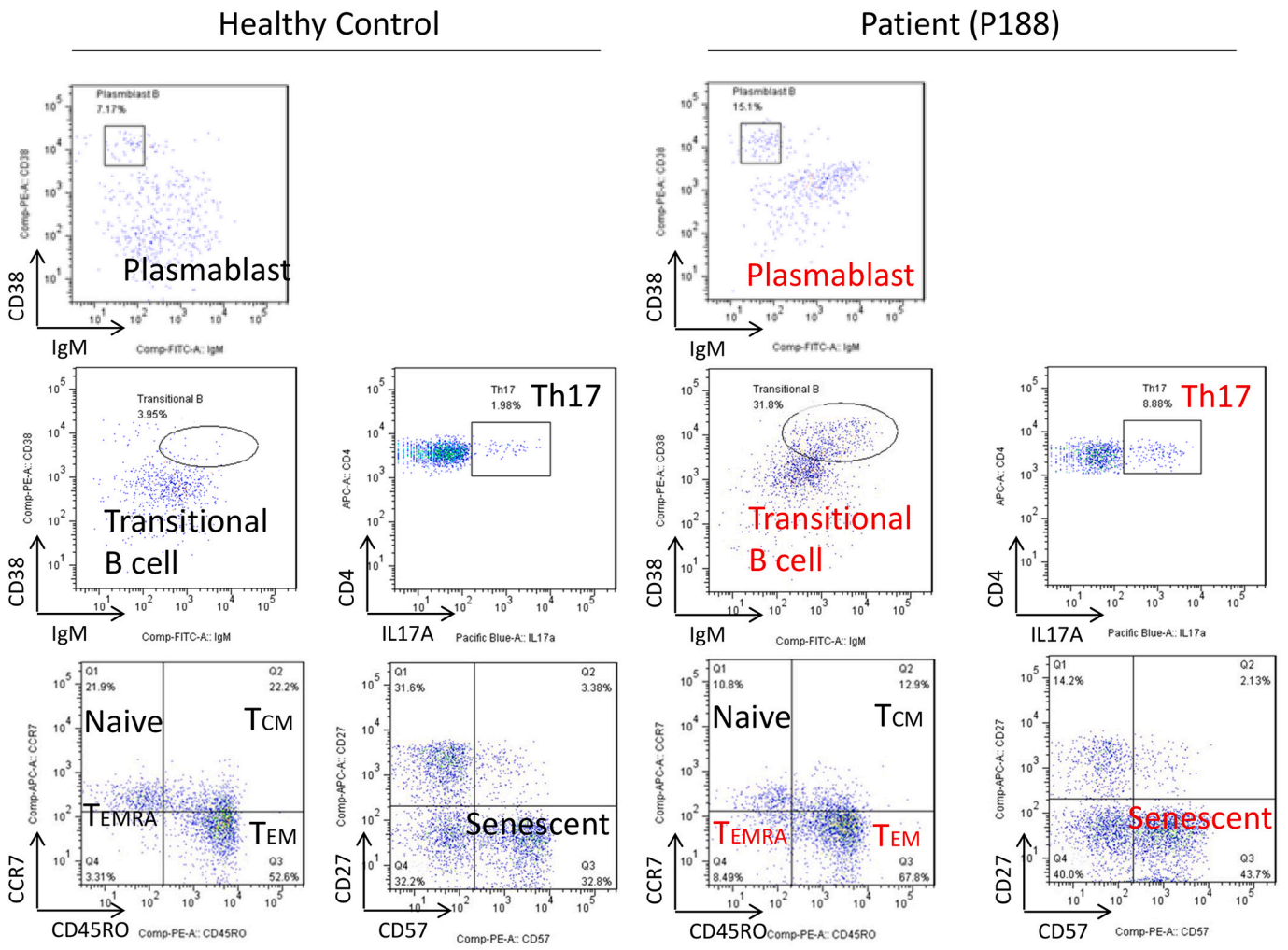

Fig. 3. Patient P188 with autoantibodies to IFN-γ had higher components of transitional B (CD38++IgM++ 31.8% vs 3.9% in the second lane), plasmablast B (CD38++IgM- 15.1% vs 7.2% in the first lane), effector CD4 memory (CD45RO + CCR7- CD4 cells; 67.8% vs 52.6% in the third lane), TEMRA (CD45RO-CCR7- CD4 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.

to persistent inflammation in CGD and T regulator dysfunction in IPEX patients.

In consistence with our previous study Treg and Th17 cells remain at a lower range in most PID patients [14], relatively higher Treg populations in patients with *PIK3R1*, *ZAP70*, *IFNG*R and *STAT3* mutations may compensate an overactive inflammation/autoimmune response. In other words, the highest Th17 component in the patients with anti-IFNγ autoantibodies implied that Th17 augmentation enhanced the higher levels of anti-IFNγ autoantibodies. As well as anti-CD20 deleting B-cell therapy (rituximab) to eradicate anti-IFNγ autoantibodies-producing B cells as far as possible [25], anti-Th17 antagonists (secukinumab) may be an additional treatment to attenuate Th17 signaling for indirectly decreasing anti-IFNγ autoantibodies.

This study should be interpreted in light of its limitations. First, the cross-sectional assessments of lymphocyte immunophenotypes at diagnosis of the LPD phenotype may have been confounded by accompanying subclinical infections. Longitudinal and large-scale follow-up of lymphocyte immunophenotypes in PID patients with LPD and comparison to those without LPD rather than merely healthy controls should more accurately reflect the whole phenotypic spectrum, although those without PID still have the potential to develop the LPD phenotype at their older ages. Second, 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, international collaboration to gather more of these respective rare PID patients for a longitudinal immunophenotyping study has more chances to validate our observation in an ethnically diverse population.

In conclusion, increased senescent CD8 + CD57+ and CD21-low components and disturbed transitional B, plasmablast B and TEMRA components were observed in our one-third Taiwanese PID patients with the LPD phenotype (32.3%, 31/96) had higher mortality. EBV infection was only detected in two (6.5%, 2/31), but unrelated to lymphoma transformation in the other two. However, in those with profound T cell defects, whether the extremely low and predominantly naïve T but absent other immunophenotyping afterwards evolved their LPD phenotype remain to be determined.

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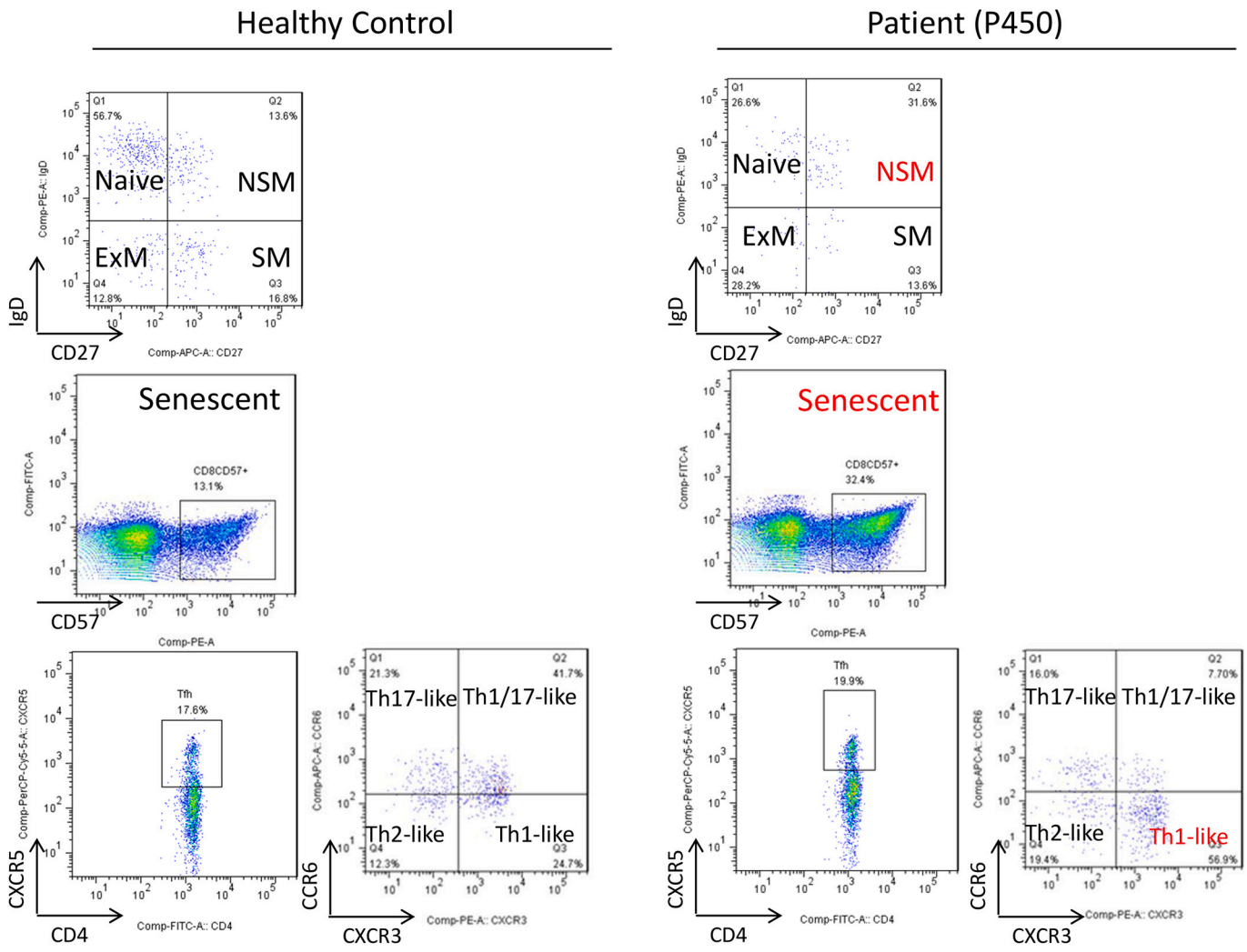

Fig. 4. The X-linked granulomatous diseases (XL-CGD) patient P450 had a higher non-class-switched memory B (IgD + CD27 + memory 31.6% vs 13.6% in the first lane) and senescent CD8 + CD57+ components (32.4% vs 13.1 in the second lane). However, the normal Tfh components (CD4 + CXCR5+; 30.8% vs 23.9% in 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.

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The authors have no financial relationships relevant to this article.

Author contributions

Lee WI, Liang CJ and Kan CC carried out the molecular genetic studies, analyzed the sequence alignment and drafted the manuscript. Lee WI and Huang JL performed the immunoassays and designed the study and the statistical analysis. Hsieh MY, Chen LC, Yeh KW, Yao TC, Ou LS, Wu CY, Lin SJ, Jaing TH, Chen SH and Huang JL participated in the study to care for critical patients. All authors read and approved the final version of the manuscript.

Ethics approval

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.

Consent to participate

Written informed consent was obtained from all individual participants were included in the study.

Consent for publication

The authors affirm that human research participants provided informed consent for publication.

CRediT authorship contribution statement

Wen-I Lee: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jing-Long Huang:** Visualization, Validation, Supervision, Formal analysis, Data curation. **Meng-Ying Hsieh:** Visualization, Validation, Supervision, Resources, Data curation. **Li-Chen Chen:** Supervision, Resources. **Kuo-Wei Yeh:** Visualization, Data curation. **Tsung-Chieh Yao:** Supervision, Data curation. **Chao-Yi Wu:**

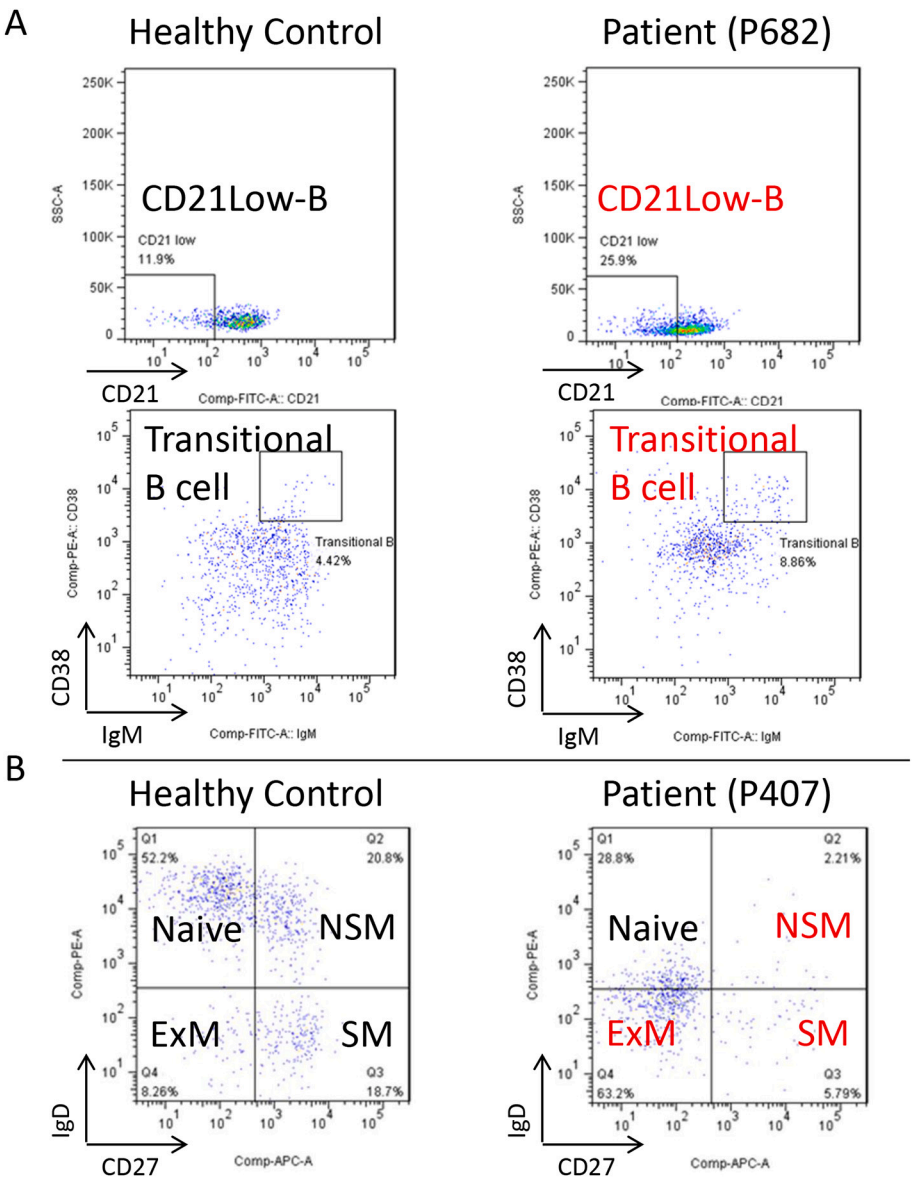

Fig. 5. The XIAP patient (P682) had higher CD21-low (25.9% vs 11.9%) and transitional B components (8.9% vs 4.4%) under CD19 gating (A). The IPEX patient (P407) had higher exhausted memory (IgD-CD27- or IgD-IgM-; 63.2% vs 8.3%) double negative cell components under CD19 gating (B).

Supervision, Formal analysis, Data curation. **Syh-Jae Lin:** Conceptualization. **Shih-Hsiang Chen:** Validation, Supervision, Resources. **Tang-Her Jaing:** Resources. **Chi-Jou Liang:** Supervision, Data curation. **Chen-Chen Kang:** Software, Methodology, Data curation.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Availability of data and material

The datasets generated during the current study are available in this article and from the corresponding author on reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2024.110269.

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