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Comprehensive phenotyping of human peripheral blood B lymphocytes in pathological conditions

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Abstract

Several diseases are associated with alterations of the B-cell compartment. Knowing how to correctly identify by flow cytometry the distribution of B-cell populations in the peripheral blood is important to help in the early diagnosis. In the accompanying article we describe how to identify the different B-cell subsets in the peripheral blood of healthy donors. Here we show a few examples of diseases that cause dysregulation of the B-cell compartment.

KEYWORDS
B-cell subsets, flow cytometry, human B cells, phenotyping

1 | INTRODUCTION

Several published reports have reported the use of complex and effective staining for B cell phenotyping [1–5]. Here we show the method we use to distinguish the B-cell subpopulations in the peripheral blood of patients with different diseases associated with B-cell perturbations. B-cell phenotyping can be useful to address diagnosis and follow-up patients during therapy. As already described (accepted doi: 10.1002/cyto.a.24507), B cells are identified as $CD19^{pos}$ and can be further classified as transitional, mature-naïve, memory, atypical memory, activated B cells and plasmablasts according to their maturation stage and function. By flow-cytometry the different subpopulations can be recognized by the regulated expression of different combinations of cluster of differentiation (CD) markers on their surface, as reported in Table 1.

To identify each B-cell population we used a combination of nine antibodies (BD, Biosciences) labeled with different fluorochrome: CD45 V500-C (2D1), CD19 PE-Cy7 (SJ25C1), CD24 PE (ML5), CD27 APC (L128), CD38 PerCP-Cy5.5 (HIT2), CD21 BV605 (B-ly4), IgM FITC (G20-127), IgG APC-H7 (G18-145), and IgD V450 (IA6-2) (Table 2). Our staining is performed on whole blood following red blood cell lysis. Bulk lysing is performed to lyse the entire blood sample to be stained. Briefly, 500 μL of fresh total peripheral blood (EDTA) are incubated for 10 min at room temperature with 9.5 mL of the lysing solution Pharm Lyse 1X (BD Biosciences) to remove red blood cells. Afterwards, cells are washed twice with 10 mL of

TABLE 1 Cluster of differentiation expressed on the different B-cell population

	Transitional	Mature-Naïve	Memory	Plasmablasts	Atypical memory	Activated memory
CD45	++	++	++	++	++	++
CD19	++	++	++	++	+++	++
CD24	+++	++	+++	−	−/+	+ and ++
CD21	+ and ++	++	++	+	−	−
CD27	−	−	+ and +++	+++	−	++
CD38	+++	+	−	+++	−	−
Igs	IgM+IgD+	IgM+IgD+	IgM+IgD+ IgG+ IgA+ IgM only IgE+	intracellular staining	IgM+IgD+ IgG+ IgM only	IgM+IgD+ IgG+

TABLE 2 Antibodies used for the staining of the peripheral blood and the identification of the B-cell population

	Fluorochrome	Clone
CD45	V500-C	2D1
CD19	PE-Cy7	SJ25C1
CD24	PE	ML5
CD27	APC	L128
CD38	PerCP-Cy5.5	HIT2
CD21	BV605	B-ly4
IgM	FITC	G20-127
IgG	APC-H7	G18-145
IgD	V450	IA6-2

phosphate-buffered saline (PBS) containing 1% of bovine serum albumin (BSA). Two washing steps after lysis are essential because they wash-off free IgG immunoglobulins in the plasma that, by sequestering the labeled anti-IgG antibody, prevent the identification of memory B cells expressing IgG on the surface. Cells are re-suspended in 200 μL of PBS and added to a B-Cell lyotube (BD, Bioscience). CD21 BV605 is added as a drop-in. After 20 min incubation at room temperature in the dark, the samples are washed in PBS 1% BSA and finally re-suspended in 300 μL of PBS 1% BSA. Data are acquired on a BD FACSLyric™ cytometer (BD Biosciences) and analyzed by FlowJo ver. 10.7 (Becton, Dickinson & Company) (See supporting informations).

Live cells are identified based on the FSC/SSC lympho-monocyte gate and then selected as $CD45^{pos}CD19^{pos}$ B cells [6]. Briefly, we first discriminate transitional B cells ($CD24^{pos}CD38^{bright}$) and plasmablasts ($CD24^{neg}CD38^{bright}$). CD21 expression separates transitional B cells into $CD21^{dull}$ transitional 1 B cells (T1, corresponding to recent bone marrow emigrants), and $CD21^{brigh}$ transitional 2 (T2). Plasmablasts are $CD21^{neg}$. We create a not transitional/not plasmablasts gate that includes all other B cell populations. We plot CD27 versus CD21 in order to identify mature-naïve ($CD27^{neg}CD21^{pos}$), memory ($CD27^{pos}CD21^{pos}$), atypical memory ($CD21^{neg}CD27^{neg}$), and activated memory B cells ($CD21^{neg}CD27^{pos}$). Based on the intensity of CD27, memory B cells can be further divided into $CD27^{dull}$ and $CD27^{bright}$ [7]. $CD27^{dull}$ are the first population of memory B cells that develop in children and can be generated independently of T cells and the germinal center reaction [7, 8], $CD27^{bright}$ cells are detected only when the cooperation between T and B cells is perfectly functional [7]. In the $CD27^{pos}$ memory B-cell population, we find IgD^{pos} and IgD^{neg} memory B cells. IgD^{neg} memory B cells can be further divided into IgG^{pos}, $IgG^{neg}IgM^{neg}$ (that are mostly IgA^{pos}), [9] and IgM-only [10]. IgD^{pos} memory B cells also express IgM and are called IgM memory B cells ($IgM^{pos}IgD^{pos}CD27^{pos}$) [11, 12]. IgM memory B cells are an heterogeneous population composed of innate memory B cells [8] and IgM memory B cells that have been remodeled in the germinal centers [13–15].

Among atypical memory B cells, IgM, and IgD expression identifies different subtypes. Atypical memory B cells increase during autoimmune disease, aging, and in viral infections [16–21]. These cells are the product of an extrafollicular or of an impaired germinal center reaction [21, 22].

A correct staining allows to identify the different B-cell populations and is of importance and utility in the diagnosis of diseases associated with these cells (Table 3).

2 | MODIFICATION OF B-CELL SUBSETS IDENTIFIED BY FLOW CYTOMETRY IN PERIPHERAL BLOOD OF PATIENTS WITH IMMUNE DISORDERS

In this brief review we provide selected examples of alterations of the B-cell phenotype in different diseases.

2.1 | Bone marrow transplantation

After hematopoietic stem cell transplantation cells of the B lineage first appear in the bone marrow where the early precursors differentiate and proliferate. Around day 30 after transplantation, rare B cells become detectable in the periphery. They are transitional B cells of the $CD21^{dull}$ type (Figure 1A) and very few plasmablasts. At day 60, the frequency of B cells increases. Most of them are still transitional B cells, now both $CD21^{dull}$ and $CD21^{pos}$. Plasmablasts are

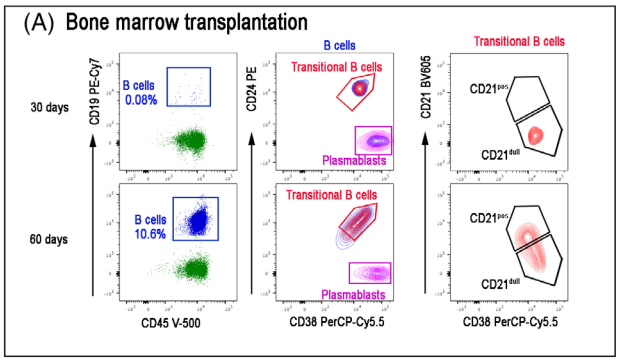
TABLE 3 Immunodeficiencies with B-cell alterations [23]

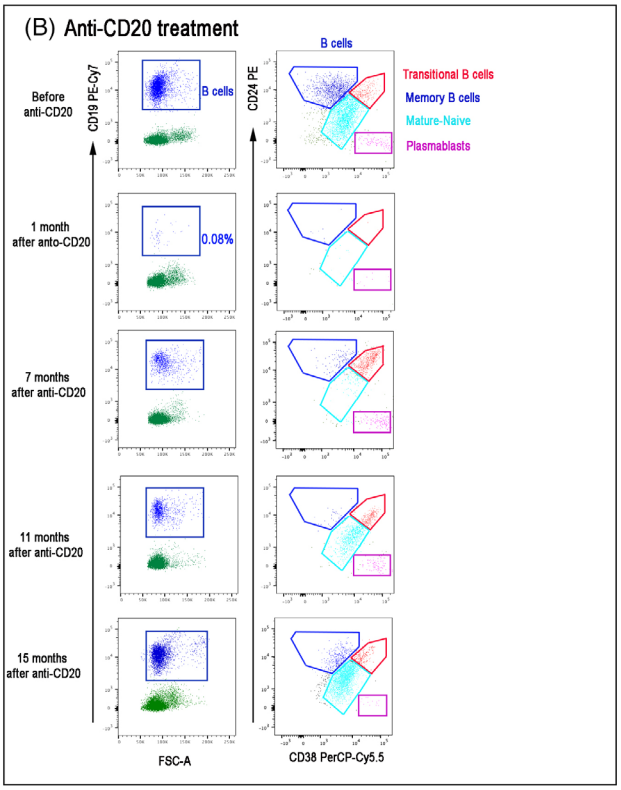
		Circulating B cells	Disturbances of B cell maturation/differentiation steps
SCID B+	X-linked SCID	Normal	Decreased memory B cells and absence switched memory B cells
	JAK3	Normal or increased	Decreased memory B cells and absence switched memory B cells
	IL7R	Normal or increased	Decreased memory B cells and absence switched memory B cells
	CD45 deficiency	Normal	Decreased switched memory B cells
	CD3δ deficiency	Normal	Decreased switched memory B cells
	CD3ε deficiency	Normal	Decreased switched memory B cells
	CD3ζ deficiency	Normal	Decreased switched memory B cells
	LAT deficiency	Normal	Decreased memory B cells, increased transitional B cells
	IkBa GOF (NFKBIA/IkB)	Elevated B cells	Low memory B cells
SCID B-	RAG1	Significantly decreased	Not detectable
	RAG2	Significantly decreased	Not detectable
	Artemis deficiency	Significantly decreased	Not detectable
	DNA-PKcs deficiency	Significantly decreased	Not detectable
	NHEJ1 deficiency	Absent	Not detectable
	DNA ligase IV deficiency	Significantly decreased	Not detectable
	AK2 deficiency (Reticular Dysgenesis)	Decreased	Not detectable
	ADA deficiency	Absent	Not detectable
	BLNK deficiency	Absent	Not detectable
	BTK deficiency	Absent	Not detectable
	IKAROS deficiency	Significantly decreased or absent	B cell acute lymphoblastic leukemia (B-ALL)
	BAFF receptor deficiency	Decreased	Decreased switched memory B cells
	Wiskott Aldrich syndrome (WAS)	Normal or decreased	Decreased memory B cells and increased transitional B cells
	CVID	Normal or decreased	Absence of memory B cells
	TACI deficiency	Normal or decreased	Decreased switched memory B cells
	ICOS deficiency	Normal (children)/ Decreased (adults)	Decreased switched memory B cells and naive B Cells
	Hyper IgM syndromes	Normal	Absence of switched memory B cells
	IgA deficiency	Normal	Absence of IgA memory B cells
	CD19 deficiency	Normal	Decreased switched memory B cells
	AT (gene ATM/ATM)	Normal	Low naive B cells, transitional B cells and memory B cells, increased atypical memory B cells
	CD40 L deficiency	Normal	Absence of switched memory B cells
	CD40 deficiency	Normal	Absence of switched memory B cells
	Di George syndrome	Normal	Decreased switched memory B cells
	DOCK8 deficiency	Normal	Significant reduction of memory B cells; switched memory B cells low
	Fischer Evans syndrome	Normal	Increased transitional B cells and atypical memory B cells, reduction of memory B cells

measurable indicating the ability of transitional B cells to differentiate into antibody producing cells (Figure 1A). In the following months mature-naïve B cells start to increase. The reconstitution of the memory B-cell pool, representing the progressive establishment and increase of antigenic experience, can take years [24].

2.2 | Anti-CD20 treatment

CD20 is a marker expressed on cells of the B lineage first at the pre-B cell stage, increased in mature-naive and memory B cells and down-regulated in plasmablasts and plasma cells. Since the FDA approval in





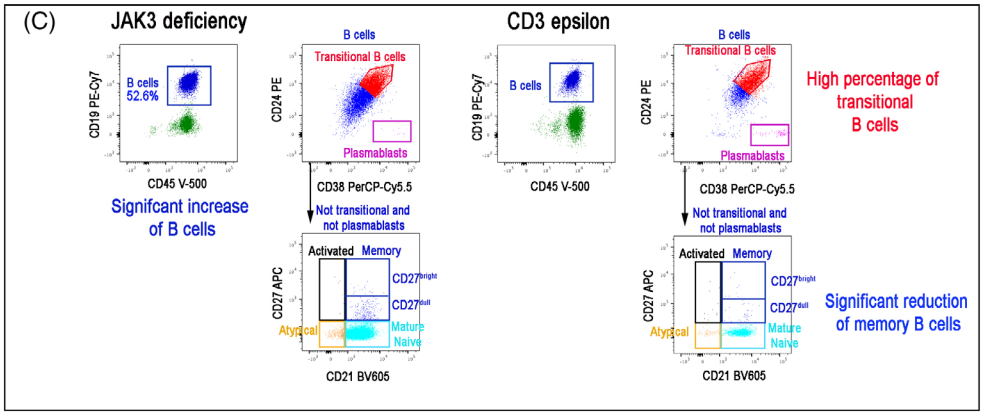


FIGURE 1 (A–B) FACS plots show the B-cell reconstitution in one patient after hematopoietic stem cell transplantation and in one patient following anti-CD20 treatment. In both patients the reconstitution starts from transitional B cells. In (C) we show FACS plots of two SCID patients, one with JAK3 deficiency and the other with CD3ε deficiency. In both patients most B cells are of the transitional and mature- naïve B-cell type and the memory B cells are strongly reduced [Color figure can be viewed at wileyonlinelibrary.com]

1997, anti-CD20 monoclonal antibodies have been successfully used in the management of B-cell lymphoma and to deplete the B-cell compartment in inflammatory and autoimmune diseases [25] . Anti-CD20 monoclonal antibodies can directly and transiently deplete all the CD20+ B-cells, including transitional, mature-naïve, and memory B-cell subsets, but can also indirectly eliminate short-lived plasma cells, which lack CD20 expression but have a limited lifespan. Peripheral CD20+ B cells usually tend to reappear after 6 months, starting with transitional and mature-naïve B cells that normalize within 12 months, whereas memory B-cell subsets remain significantly reduced for more

than 2 years from anti-CD20 administration [26–29]. Of note, the time of the B-cell subset reappearance is comparable following treatment with anti-CD20 monoclonal antibodies directed against different epitopes of CD20 such as rituximab and ofatumumab [30].

In order to evaluate the duration of B-cell depletion and follow-up the reconstitution of the B-cell compartment in treated patients, a combined B and T cell staining has been proposed [31]. Our staining can be also used to monitor B cell depletion and regeneration (Figure 1B). Here we show the phenotype of peripheral B cells in the same individual before and up to 15 months after one single anti-CD20 monoclonal antibody dose. The patient was a 13 year-old child with steroid-dependent nephrotic syndrome. As after hematopoietic stem cell transplantation, the reconstitution starts from transitional B cells. Also in this case, the regeneration of the B-cell memory pool is very slow.

2.3 | Severe combined immunodeficiency

Severe combined immunodeficiency (SCID) includes a group of rare disorders caused by mutations in different genes involved in the development and function of the immune system. Based on the immunological phenotype and the underlying genetic defect, different types of SCID can be classified (Table 3): without T cells but with B cells (T-B+ SCID) or SCID without T and B cells (T-B− SCID). Both of these groups include several forms with or without natural killer (NK) cells [32].

SCID typically presents in infancy with early-onset severe respiratory infections and failure to thrive and, without therapy, leads to death within the first year of life. Hematopoietic stem cell transplantation is the curative treatment. In Figure 1B, we show two examples of B-cell development in JAK3 and CD3ε deficiency, two autosomal recessive forms of SCID [32].

The first patient, a 1-year-old boy, had a mutation of the JAK3 tyrosine kinase that is indispensable for signals generated by all interleukin receptors containing the gamma chain (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) [33]. As IL-7 is indispensable for the development of T cells and IL-15 is necessary for the production of NK cells, patients with JAK3 mutations have no T and NK cells. IL-7, which regulates B cell development in the mouse, does not have this function in humans. The mutation abolishes the T and NK cell compartments, whereas B cells are generated in the bone marrow, resulting in a T-B+NK− phenotype. In the peripheral blood most B cells are of the transitional and mature B-cell type. Memory B cells are all $CD27^{dull}$ (Figure 1C) and only express IgM [7, 8].

The T-cell antigen receptor (TCR) and pre-TCR complexes are composed of multiple signal-transducing subunits (CD3γ, CD3δ, CD3ε, and ζ). Signaling by the TCR and its precursor, the pre-TCR, are required for T-cell development and selection. Patients with CD3ε deficiency have no T cells, but B and NK cells are in normal or increased numbers (T-B+NK+ phenotype) [32].

Also in this patient, analyzed and diagnosed at 8 months of life, most of the B cells have the transitional or mature-naïve phenotype (Figure 1C). Also in this patient, the few memory B cells developing in absence of T cells are $CD27^{dull}$ IgM memory B cells [7, 8].

2.4 | Common variable immunodeficiency

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency (PID). It is characterized by hypogammaglobulinemia and impaired production of specific antibodies. Patients with CVID present a broad range of clinical manifestations, including recurrent bacterial infections, autoimmunity, interstitial lung disease, enteropathy, lymphoproliferation, malignancy, and allergic diseases [34]. In most cases the genetic cause of disease remains unknown, but monogenic disorders [35] and epigenetic modifications have been identified as responsible for CVID [36, 37]. Since CVID includes a heterogeneous group of primary antibody immunodeficiencies with various clinical and immunological features, in recent years, several classifications have been proposed including a discrimination based on the phenotype of B-cell populations [38]. In Figure 2A we show the B-cell phenotype of a 14 years old CVID patient with a significant reduction of transitional and mature-naive B cells and an increased frequency of atypical memory B cells. In this particular patient all memory B cells are $CD27^{dull}$ suggesting an impairment of the germinal center reaction [7]. $CD27^{dull}$ memory B cells are either $IgM^{pos}IgD^{pos}$ or IgM only with a complete absence of switched memory B cells thus confirming the inability of this patient to carry on and complete the germinal center reaction [7, 8]. The absence or the significant reduction of memory B cells is an indicator of a subgroup of CVID patients with a severe prognosis [39], and predicts clinical prognosis more accurately than serum immunoglobulin concentrations [40]. In this regard it is interesting to note that the reduction of memory B cells might be a predictive marker of outcome in hypogammaglobulinemia during infancy [41].

2.5 | Ataxia telangiectasia

Ataxia telangiectasia (AT) is an autosomal-recessive primary immunodeficiency disorder caused by genetic mutation in the ataxia telangiectasia mutated (ATM) gene, located on chromosome 11q22-23, which is involved in the recognition and repair of damaged. AT is characterized by ataxia, neurologic decline, oculocutaneous telangiectasia, a wide range of immune function abnormalities [42, 43] and increased risk of developing various types of cancer [44]. AT patients present with recurrent viral and bacterial infections, which cause significant morbidity and are a frequent factor or cause of death [45]. Signs of T and B immunodeficiency of variable severity have been reported: decrease of naïve CD4+ and CD8+ T lymphocytes, increase of NK cells and reduced numbers of B cells [46, 47]. Among the dysfunctions in immunoglobulin production, increased levels of IgM, reduction of IgG, IgA deficiency, and lack of response to polysaccharide antigens are the most frequent [46]. DNA repair activity is indispensable in the bone marrow for immunoglobulin recombination and in the germinal

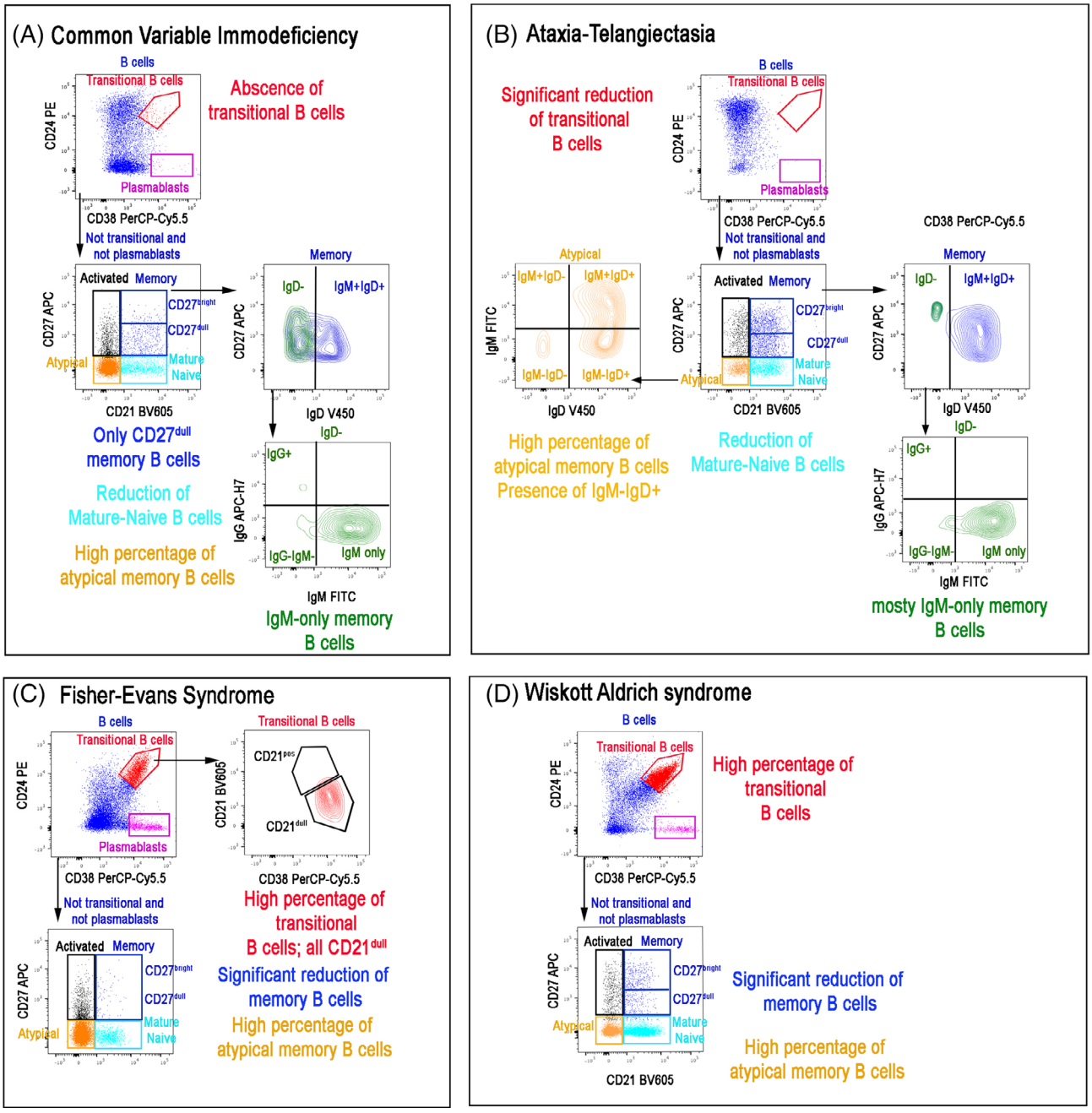


FIGURE 2 FACS plots show the B-cell population in four examples of immune disorders. CVID (A) and ataxia-telangiectasia (B) patients have both a significant reduction of transitional B cells and mature-naïve B cells and a higher frequency of atypical memory B cells. The CVID patient only has few CD27^{dull} memory B cells that express either IgD and IgM or IgM only, thus lacking switched memory B cells. Patients with Fisher-Evans Syndrome (C) and with Wiskott Aldrich syndrome (D) present a significantly higher number of transitional B cells, an increased frequency of atypical memory B cells and a strong reduction of memory B cells [Color figure can be viewed at wileyonlinelibrary.com]

centers for the mechanisms of somatic mutation and class switch recombination. All these processes are severely impaired in AT [47]. The FACS-plots of a 18-year-old AT patient are depicted in Figure 2B. The reduction of transitional and naive B cells is the consequence of the poor production of B cells in the bone marrow due to the inefficient process of immunoglobulin rearrangement, whereas the low number of switched memory B cells and the increased frequency of atypical memory B cells can be explained by the impairment of somatic mutation and class-switch recombination in the germinal centers [48].

2.6 | Fisher-Evans'syndrome

Fisher-Evans'syndrome (ES) is defined as the concomitant or sequential association of auto-immune haemolytic anemia (AIHA)

with immune thrombocytopenia (ITP), and less frequently autoimmune neutropenia. ES is a rare situation that represents up to 7% of AIHA and around 2% of ITP [49, 50]. The determination of the primary or secondary nature of ES is important, since the association of this syndrome with other diseases, such as hematological malignancies, systemic lupus erythematosus, infections, or primary immune deficiencies (PID) can interfere with the management or alter the prognosis [50]. Inborn errors of immunity were reported in about 70% of ES patients and recently the new sequencing techniques allowed the discovery of several novel mutations [51, 52]. In these patients specific immunophenotypic characteristics were identified, such as T and B lymphopenia, decrease in naïve T cells, switched memory B cells, plasmablasts, and/or immunoglobulins, increase in effector/central memory T-cells and increase of CD21low atypical B memory cells [53]. In Figure 2C we show the immune phenotype of a 17-year-old patient with ES characterized by high frequencies of transitional B cells, that are all CD27^{dull}, and of atypical memory B cells, and a significant reduction of memory B cells.

2.7 | Wiskott–Aldrich syndrome

Wiskott–Aldrich syndrome (WAS) is an X-linked disease caused by mutations in the *WAS* gene characterized by a triad of thrombocytopenia, eczema, and immunodeficiency [54]. Without curative therapies, affected patients have diminished life expectancy and reduced quality of life. The WAS protein (WASP) is constitutively expressed in all hematopoietic stem cell-derived lineages and is located predominantly in the cytoplasm with the highest protein expression toward the cell membrane [55]. WASP is a member of a distinct family of proteins that link signaling pathways to actin cytoskeleton reorganization and localize in the immunologic synapse [56], the site of the interaction between various leukocyte subpopulations. The immunological synapse allows the physical contact between antigen presenting cells, T and B cells and it is required for an adaptive immune response, including class switch recombination and somatic hypermutation [55, 57]. WAS is associated with autoimmunity and increased incidence of hematologic malignancies, especially B-cell lymphomas. Affected patients are susceptible to bacterial, viral, and fungal infections,

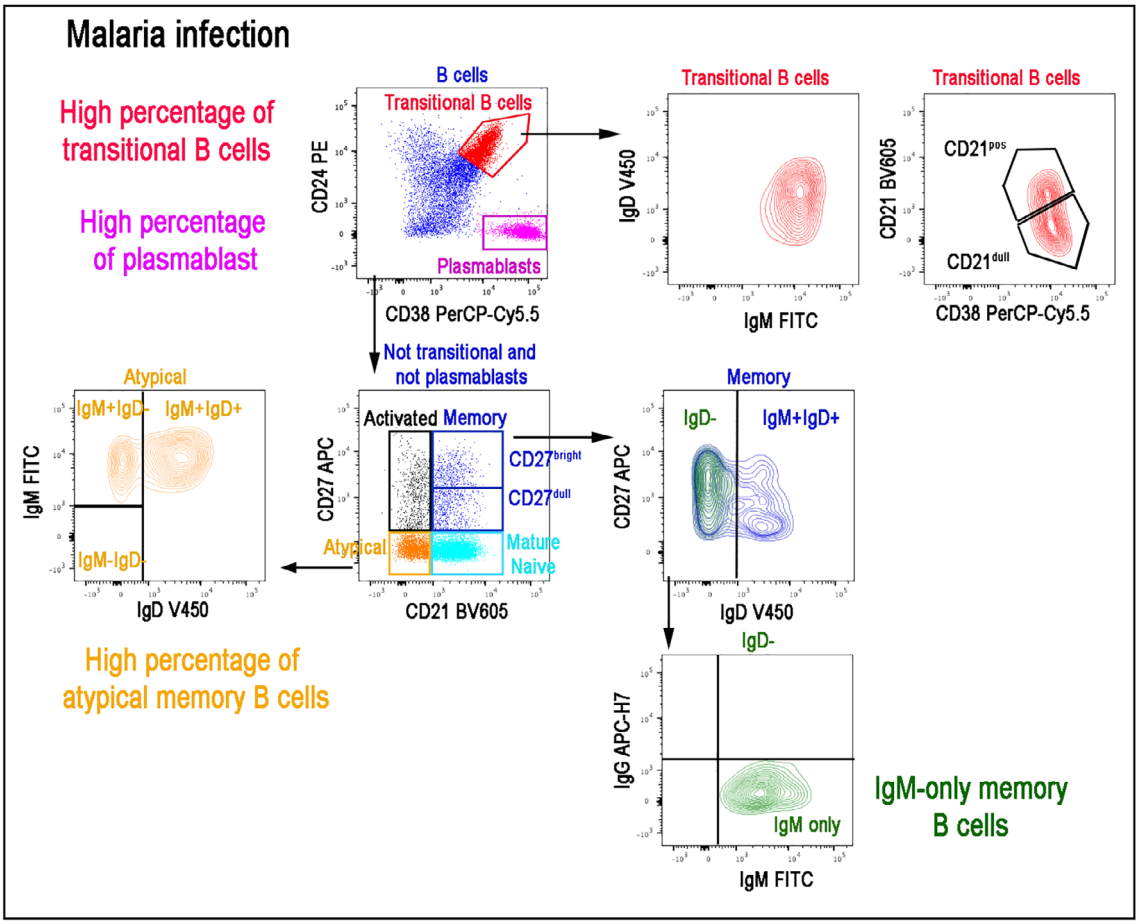


FIGURE 3 FACS plots showing the B-cell population in a patient with malaria. This patient shows an increased frequency of transitional B cells, plasmablasts and atypical memory B cells. All CD27^{pos}IgD^{neg} memory B cells are IgM-only [Color figure can be viewed at wileyonlinelibrary.com]

secondary to defects in both cellular and humoral immunity. In the Figure 2D the B-cell phenotype of a 6-year-old child shows a prevalence of transitional B cells and atypical memory B cells associated with a reduction of the memory pool. The altered distribution of the B-cell populations might be explained by the impairment of adaptive immune responses in the germinal centers.

2.8 | *Plasmodium falciparum* infection

B cells and antibodies are critical for control of parasitemia in malaria, caused by *P. falciparum* infection. Antibodies also provide protection to reinfection. However, it has been demonstrated that in the absence of constant re-exposure, Plasmodium-specific serum antibody levels rapidly decrease, and full protection from clinical symptoms is lost, suggesting that B-cell memory is functionally impaired [58]. Some studies attribute this to the expansion of atypical memory B cells, which express multiple inhibitory receptors and activation markers, and are hyporesponsive to B-cell receptor (BCR) restimulation in vitro [59]. In Figure 3 the B-cell phenotype of a 3-year-old child diagnosed with *P. falciparum* malaria shows expansion of plasmablasts, transitional B cells and atypical memory B cells in the peripheral blood. The phenotype of this patient is also characterized by the absence of IgGpos and IgApos switched memory B cells.

In conclusion, our staining identifies different B-cell populations that have distinct phenotype and precise functions. A proper B-cell flow cytometric analysis, that correctly identifies the different B-cell populations in the peripheral blood, may address the diagnosis in immunodeficiency and be useful in the follow-up in different clinical conditions.

AUTHOR CONTRIBUTIONS

Francesco Corrente: Formal analysis (equal); methodology (equal). **Claudia Capponi:** Formal analysis (equal); methodology (equal). **Mattia Mirabella:** Methodology (equal). **Simona Cascioli:** Methodology (equal). **Patrizia Palomba:** Methodology (equal). **Valentina Bertaina:** Methodology (equal). **Manuela Colucci:** Formal analysis (equal); methodology (equal).

PEER REVIEW

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REFERENCES

1. Liechti T, Günthard HF, Trkola A. OMIP-047: high-dimensional phenotypic characterization of B cells. Cytometry Part A. 2018;93:592–6. https://doi.org/10.1002/cyto.a.23488
2. Liechti T, Roederer M. OMIP-051 – 28-color flow cytometry panel to characterize B cells and myeloid cells. Cytometry Part A. 2019;95:150–5. https://doi.org/10.1002/cyto.a.23689
3. Cascino K, Roederer M, Liechti T. OMIP-068: high-dimensional characterization of global and antigen-specific B cells in chronic infection. Cytometry Part A. 2020;97:1037–43. https://doi.org/10.1002/cyto.a.24204
4. Streitz M, Miloud T, Kapinsky M, Reed MR, Magari R, Geissler EK, et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. Transplant Res. 2013;2:17. https://doi.org/10.1186/2047-1440-2-17
5. van Dongen JJM, van der Burg M, Kalina T, Perez-Andres M, Mejstrikova E, Vlkova M, et al. EuroFlow-based Flowcytometric diagnostic screening and classification of primary Immunodeficiencies of the lymphoid system. Front Immunol. 2019;10:1271. https://doi.org/10.3389/fimmu.2019.01271
6. Carsetti R, Terreri S, Conti MG, Fernandez Salinas A, Corrente F, Capponi C, et al. Comprehensive phenotyping of human peripheral blood B lymphocytes in healthy conditions. Cytometry Part A. 2021;1–9. https://doi.org/10.1002/cyto.a.24507
7. Grimsholm O, Mortari EP, Davydov AN, Shugay M, Obraztsova AS, Bocci C, et al. The interplay between CD27dull and CD27bright B cells ensures the flexibility, stability, and resilience of human B cell memory. Cell Rep. 2020;30:2963–2977.e6.
8. Aranburu A, Piano Mortari E, Baban A, Giorda E, Cascioli S, Marcellini V, et al. Human B-cell memory is shaped by age- and tissue-specific T-independent and GC-dependent events. Eur J Immunol. 2017;47:327–44.
9. Carsetti R, Zaffina S, Piano Mortari E, Terreri S, Corrente F, Capponi C, et al. Different innate and adaptive immune responses to SARS-CoV-2 infection of asymptomatic, mild, and severe cases. Front Immunol. 2020;11.3365–3381.
10. Bagnara D, Squillario M, Kipling D, Mora T, Walczak AM, da Silva L, et al. A reassessment of IgM memory subsets in humans. J Immunol. 2015;195:3716–24. https://doi.org/10.4049/jimmunol.1500753
11. Seifert M, Przekopowitz M, Taudien S, Lollies A, Ronge V, Drees B, et al. Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions. Proc Natl Acad Sci. 2015;112:E546–55. https://doi.org/10.1073/pnas.1416276112
12. Berkowska MA, Driessen GJA, Bikos V, Grosserichter-wagener C, Cerutti A, He B, et al. Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways human memory B cells originate from three distinct germinal center- dependent and -independent maturation pathways. Blood. 2011;118:2150–9.
13. Aranburu A, Mortari EP, Baban A, Giorda E, Cascioli S, Marcellini V, et al. Human B-cell memory is shaped by age- and tissue-specific T-independent and GC- dependent events. Eur J Immunol. 2016;47:10–1.
14. Budeus B, Schweigle de Reynoso S, Przekopowitz M, Hoffmann D, Seifert M, Küppers R. Complexity of the human memory B-cell compartment is determined by the versatility of clonal diversification in germinal centers. Proc Natl Acad Sci. 2015;112:E5281–9. https://doi.org/10.1073/pnas.1511270112
15. Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, et al. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood. 2004;104:3647–54.
16. Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, Kappler JW, et al. Toll-like receptor 7 (TLR7)–driven accumulation of a novel CD11c+ B-cell population is important for the development of autoimmunity. Blood. 2011;118:1305–15. https://doi.org/10.1182/blood-2011-01-331462
17. Wang S, Wang J, Kumar V, Karnell JL, Naiman B, Gross PS, et al. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11chiT-bet+ B cells in SLE. Nat Commun. 2018;9:1758.
18. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, et al. Distinct effector B cells induced by unregulated toll-like receptor 7 contribute to pathogenic responses in systemic lupus Erythematosus. Immunity. 2018;49:725–739.e6. https://www.sciencedirect.com/science/article/pii/S1074761318303789

19. Rubtsova K, Rubtsov AV, van Dyk LF, Kappler JW, Marrack P. T-box transcription factor T-bet, a key player in a unique type of B-cell activation essential for effective viral clearance. Proc Natl Acad Sci. 2013;110:E3216 LP–E3224. http://www.pnas.org/content/110/34/E3216.abstract
20. Knox JJ, Buggert M, Kardava L, Seaton KE, Eller MA, Canaday DH, et al. T-bet+ B cells are induced by human viral infections and dominate the HIV gp140 response. JCI Insight. 2017;2:e92943. https://pubmed.ncbi.nlm.nih.gov/28422752
21. Oliviero B, Varchetta S, Mele D, Mantovani S, Cerino A, Perotti CG, et al. Expansion of atypical memory B cells is a prominent feature of COVID-19. Cell Mol Immunol. 2020;17:1101–3.
22. Ubillos I, Campo JJ, Requena P, Ome-Kaius M, Hanieh S, Rose H, Samol P, Barrios D, Jiménez A, Bardají A, Mueller I, Menéndez C, Rogerson S, Moncunill G, Dobaño C. Corrigendum: chronic exposure to malaria is associated with inhibitory and activation markers on atypical memory B cells and marginal zone-like B cells. Front Immunol 2019;10:2427. https://www.frontiersin.org/article/10.3389/fimmu.2019.02427.
23. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020;40:24–64. https://pubmed.ncbi.nlm.nih.gov/31953710
24. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune reconstitution after allogeneic hematopoietic stem cell transplantation. Front Immunol. 2016;7:507. https://doi.org/10.3389/fimmu.2016.00507
25. Casan JML, Wong J, Northcott MJ, Opat S. Anti-CD20 monoclonal antibodies: reviewing a revolution. Hum Vaccin Immunother. 2018;14:2820–41. https://pubmed.ncbi.nlm.nih.gov/30096012
26. Anolik JH, Barnard J, Owen T, Zheng B, Kemshetti S, Looney RJ, et al. Delayed memory B cell recovery in peripheral blood and lymphoid tissue in systemic lupus erythematosus after B cell depletion therapy. Arthritis Rheumat. 2007;56:3044–56. https://doi.org/10.1002/art.22810
27. Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JCW. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. Arthritis Rheumat. 2006;54:613–20. https://doi.org/10.1002/art.21617
28. Colucci M, Carsetti R, Cascioli S, Casiraghi F, Perna A, Ravà L, et al. B cell reconstitution after rituximab treatment in idiopathic nephrotic syndrome. J Am Soc Nephrol. 2016;27:1811–22. http://jasn.asnjournals.org/content/27/6/1811.abstract
29. Colucci M, Carsetti R, Rosado MM, Cascioli S, Bruschi M, Candiano G, et al. Atypical IgM on T cells predict relapse and steroid dependence in idiopathic nephrotic syndrome. Kidney Int. 2019;96:971–82. https://www.sciencedirect.com/science/article/pii/S0085253819304612
30. Ravani P, Colucci M, Bruschi M, Vivarelli M, Cioni M, DiDonato A, et al. Human or chimeric monoclonal anti-CD20 antibodies for children with Nephrotic syndrome: a superiority randomized trial. J Am Soc Nephrol. 2021;32:2652–63. http://jasn.asnjournals.org/content/32/10/2652.abstract
31. Gatti A, Buccisano F, Scupoli MT, Brando B. The ISCCA flow protocol for the monitoring of anti-CD20 therapies in autoimmune disorders. Cytometry Part B: Clin Cytomet. 2021;100:194–205. https://doi.org/10.1002/cyto.b.21930
32. Candotti F, Villartay J-P, Moshous D, Villa A, Notarangelo LD. Chapter 7 - severe combined immune deficiency. In: Sullivan KE, Stiehm ER, editors. Stiehm's immune deficiencies. 2nd ed. Cambridge, Massachusetts: Academic Press; 2020. p. 153–205. https://www.sciencedirect.com/science/article/pii/B9780128167687000077
33. Notarangelo LD, Bacchetta R, Casanova J-L, Su HC. Human inborn errors of immunity: an expanding universe. Sci Immunol. 2020;5:eabb1662. https://pubmed.ncbi.nlm.nih.gov/32651211
34. Park MA, Li JT, Hagan JB, Maddox DE, Abraham RS. Common variable immunodeficiency: a new look at an old disease. Lancet (London, England). 2008;372:489–502.
35. Yazdani R, Abolhassani H, Kiaee F, Habibi S, Azizi G, Tavakol M, et al. Comparison of common monogenic defects in a large predominantly antibody deficiency cohort. J Allergy Clin Immunol Pract. 2019;7:864–878.e9. https://www.sciencedirect.com/science/article/pii/S2213219818305798
36. Rae W. Indications to epigenetic dysfunction in the pathogenesis of common variable immunodeficiency. Arch Immunol Therap Experiment. 2017;65:101–10. https://doi.org/10.1007/s00005-016-0414-x
37. Rodríguez-Cortez VC, del Pino-Molina L, Rodríguez-Ubreva J, Ciudad L, Gómez-Cabrero D, Company C, et al. Monozygotic twins discordant for common variable immunodeficiency reveal impaired DNA demethylation during naïve-to-memory B-cell transition. Nat Commun. 2015;6:7335. https://pubmed.ncbi.nlm.nih.gov/26081581
38. Rösel AL, Scheibenbogen C, Schliesser U, Sollwedel A, Hoffmeister B, Hanitsch L, et al. Classification of common variable immunodeficiencies using flow cytometry and a memory B-cell functionality assay. J Allergy Clin Immunol. 2015;135:198–208.e5. https://doi.org/10.1016/j.jaci.2014.06.022
39. Salzer U, Warnatz K, Peter HH. Common variable immunodeficiency - an update. Arthritis Res Therapy. 2012;14:223. https://doi.org/10.1186/ar4032
40. Plebani A, Lougaris V, Soresina A, Meini A, Zunino F, Losi CG, et al. A novel immunodeficiency characterized by the exclusive presence of transitional B cells unresponsive to CpG. Immunology. 2007;121:183–8. https://pubmed.ncbi.nlm.nih.gov/17313486
41. Moschese V, Graziani S, Avanzini MA, Carsetti R, Marconi M, la Rocca M, et al. A prospective study on children with initial diagnosis of transient hypogammaglobulinemia of infancy: results from the Italian Primary Immunodeficiency Network. Int J Immunopathol Pharmacol. 2008;21:343–52.
42. Perry TL, Yong VW, Ito M, Foulks JG, Wall RA, Godin DV, et al. Nigrostriatal dopaminergic neurons remain undamaged in rats given high doses of l-DOPA and Carbidopa chronically. J Neurochem. 1984;43:990–3. https://doi.org/10.1111/j.1471-4159.1984.tb12834.x
43. Roifman CM, Lederman HM, Lavi S, Stein LD, Levison H, Gelfand EW. Benefit of intravenous IgG replacement in hypogammaglobulinemic patients with chronic sinopulmonary disease. Am J Med. 1985;79:171–4. https://www.sciencedirect.com/science/article/pii/0002934385900063
44. Swift M, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia–telangiectasia. N Engl J Med. 1991;325:1831–6. https://doi.org/10.1056/NEJM199112263252602
45. McKinnon PJ. ATM and ataxia telangiectasia. EMBO Rep. 2004;5:772–6. https://doi.org/10.1038/sj.embor.7400210
46. Nowak-Wegrzyn A, Crawford TO, Winkelstein JA, Carson KA, Lederman HM. Immunodeficiency and infections in ataxia-telangiectasia. J Pediatr. 2004;144:505–11. https://www.sciencedirect.com/science/article/pii/S0022347604000435
47. Driessen GJ, IJspeert H, Weemaes CMR, Haraldsson Á, Trip M, Warris A, et al. Antibody deficiency in patients with ataxia telangiectasia is caused by disturbed B- and T-cell homeostasis and reduced immune repertoire diversity. J Aller Clin Immunol. 2013;131:1367–1375.e9. https://www.sciencedirect.com/science/article/pii/S0091674913003126
48. Braddom AE, Batugedara G, Bol S, Bunnik EM. Potential functions of atypical memory B cells in plasmodium-exposed individuals. Int J

Parasitol. 2020;50:1033–42. https://www.sciencedirect.com/science/article/pii/S0020751920302575

49. Jaime-Pérez JC, Aguilar-Calderón PE, Salazar-Cavazos L, Gómez-Almaguer D. Evans syndrome: clinical perspectives, biological insights and treatment modalities. J Blood Med. 2018;9:171–84. https://pubmed.ncbi.nlm.nih.gov/30349415
50. Audia S, Grienay N, Mounier M, Michel M, Bonnotte B. Evans' syndrome: from diagnosis to treatment. J Clin Med. 2020;9:3851. https://pubmed.ncbi.nlm.nih.gov/33260979
51. Rivalta B, Zama D, Pancaldi G, Facchini E, Cantarini ME, Miniaci A, et al. Evans syndrome in childhood: long term follow-up and the evolution in primary immunodeficiency or rheumatological disease. Front Pediatr. 2019;7:304. https://doi.org/10.3389/fped.2019.00304
52. Hadjadj J, Aladjidi N, Fernandes H, Leverger G, Magérus-Chatinet A, Mazerolles F, et al. Pediatric Evans syndrome is associated with a high frequency of potentially damaging variants in immune genes. Blood. 2019;134:9–21. https://www.sciencedirect.com/science/article/pii/S0006497120424358
53. Zama D, Conti F, Moratti M, Cantarini ME, Facchini E, Rivalta B, et al. Immune cytopenias as a continuum in inborn errors of immunity: an in-depth clinical and immunological exploration. Immun Inflammat Dis. 2021;9:583–94. https://doi.org/10.1002/iid3.420
54. Massaad MJ, Ramesh N, Geha RS. Wiskott-Aldrich syndrome: a comprehensive review. Ann NY Acad Sci. 2013;1285:26–43. https://doi.org/10.1111/nyas.12049
55. Mallhi KK, Petrovic A, Ochs HD. Hematopoietic stem cell therapy for Wiskott-Aldrich syndrome: improved outcome and quality of life. J Blood Med. 2021;12:435–47. https://pubmed.ncbi.nlm.nih.gov/34149291
56. Thrasher AJ. Wasp in immune-system organization and function. Nat Rev Immunol. 2002;2:635–46. https://doi.org/10.1038/nri884
57. Bonilla FA, Oettgen HC. Adaptive immunity. J Allergy Clin Immunol. 2010;125:S33–40. https://doi.org/10.1016/j.jaci.2009.09.017
58. Portugal S, Tipton CM, Sohn H, Kone Y, Wang J, Li S, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife. 2015;4:e07218.
59. Pérez-Mazliah D, Ndungu FM, Aye R, Langhorne J. B-cell memory in malaria: myths and realities. Immunolog Rev. 2020;293:57–69. https://doi.org/10.1111/imr.12822

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