Allergol Immunopathol (Madr). 2016;44(6):571–579

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

# Variations of B cell subpopulations in peripheral blood of healthy Mexican population according to age: Relevance for diagnosis of primary immunodeficiencies

L. Berrón-Ruíz[a,b], G. López-Herrera[b], C.E. Ávalos-Martínez[c,†], C. Valenzuela-Ponce[a], E. Ramírez-SanJuan[d], G. Santoyo-Sánchez[e], F. Mújica Guzmán[f], F.J. Espinosa-Rosales[b], L. Santos-Argumedo[a,*]

[a] *Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados-IPN, México, D.F., Mexico*
[b] *Unidad de Investigación en Inmunodeficiencias, Instituto Nacional de Pediatría-SSa, México, D.F., Mexico*
[c] *Laboratorio de Inmunoquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México, D.F., Mexico*
[d] *Laboratorio de Farmacología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México, D.F., Mexico*
[e] *Programa de Posgrado en Ciencias Médicas, Odontológicas y de la Salud, Universidad Nacional Autónoma de México, México, D.F., Mexico*
[f] *Laboratorio de Hematología, Instituto Nacional de Pediatría-SSa, México, D.F., Mexico*

Received 11 January 2016; accepted 4 May 2016
Available online 22 October 2016

**KEYWORDS**
Transitional B cells;
Naïve B cells;
Memory B cells;
Plasmablasts;
Subpopulation B cells

**Abstract**
*Background:* Peripheral blood B cells include lymphocytes at various stages of differentiation, each with a specific function in the immune response. All these stages show variations in percentage and absolute number throughout human life. The numbers and proportions of B subpopulation are influenced by factors such as gender, age, ethnicity, and lifestyle. This study establishes reference values according to age of peripheral blood B cell subtypes in healthy Mexican population.
*Methods:* Peripheral blood from healthy new-borns and adults were analysed for total B cell subpopulations, using surface markers such as CD19, IgM, IgD, CD21, CD24, CD27, and CD38, to identify naïve, memory with and without isotype switch, double-negative, transitional, and plasmablast cells.
*Results:* We observed a significant variation in terms of frequency and absolute counts between all groups analysed. Values from each B cell subpopulation show variations according to age.

* Corresponding author.
*E-mail address:* lesantos@cinvestav.mx (L. Santos-Argumedo).
† CE Ávalos-Martínez, DSc, *Rest in Peace, June 25, 2014.*

http://dx.doi.org/10.1016/j.aller.2016.05.003

*Conclusions:* In order to attempt to elucidate reference values for B cell subpopulation, the present study evaluated a population sample of healthy blood donors from this region. Values reported here can also be used as a tool for diagnosis of diseases in which B cell maturation is affected.


## Introduction

Human peripheral blood cell counts may vary among healthy individuals according to age. These variations may also be due to pathologies that involve the immune system, i.e., primary immunodeficiency (PIDs).[1] B cell differentiation is a highly regulated process that varies according to age; in embryonic development, human B cells are generated in fetal liver; from new-borns to adults, early B cell development occurs in bone marrow, whereas differentiation to generate mature B cells, memory, or plasma cells occurs in peripheral lymphoid organs such as the spleen or lymph nodes. Peripheral B cells include immature B cells, also called transitional B cells, a population that recently emigrated from bone marrow; these cells are in transit to peripheral lymphoid organs such as the spleen or lymph nodes where they fulfill their maturation.[2,3] Antigen recognition by follicular B cells together with co-stimulation received from follicular helper T cells induces class-switch recombination and B cell differentiation to generate memory B cells. These cells are characterised by the loss of IgD expression, the presence of either IgM, IgG, or IgA and are usually identified by the surrogate marker CD27, are long-lived and, upon reencounter with antigen, differentiate into antibody-producing cells (plasma cells).[3]

PIDs have a calculated incidence of 1–5 patients per 100,000 habitants. Annually, the European and Latin-American societies for immunodeficiencies (ESID and LASID, respectively) report at least 1000 new cases for PIDs.[4] The statistics in LASID show that in a single national health institute at least 30 new PIDs cases were identified per month,[5,6] indicating that the calculated incidence might be underestimated. Common Variable Immune Deficiency (CVID) is, after IgA deficiency, the most frequently diagnosed PID. CVID is characterised by low immunoglobulin levels (at least two of the main isotypes are decreased) due to alterations in peripheral B cell development.[7] As CVID is diagnosed at two peaks of age (children and adults; it is important to have reference ranges of all B cell subpopulations in peripheral blood.[8] Three major classifications of CVID have been proposed, all of them based on the impairment of the B cell phenotypes.[9–11] To predict associated clinical complications, we used Freiburg Classification; this classification is based on the decrease of memory B cells and reported that the expansion of B cells CD21$^{low}$ subpopulation allows the detection of patients with the highest risk to develop splenomegaly and/or granuloma.[10] Additionally, most PIDs are diagnosed during childhood.[5] Several primary immunodeficiencies have shown impairment of central or peripheral B cell development, such as X-linked agammaglobulinaemia (XLA), CVID, or X-linked lymphoproliferative disease (XLP); these patients have a defective differentiation of precursors (XLA) or mature B cells (XLP).[7]

Immunophenotyping by flow cytometry has been used to delineate the stages of peripheral B cell maturation in healthy population with the purpose of establishing reference values according to age, and to create the basis for correlating the clinical data and the laboratory findings from patients with immunological diseases.[12,13] It has been demonstrated that lymphocytes subsets vary significantly depending on ethnicity, exposure to infectious disease, environmental factors and age.[14] For that reason, we analysed peripheral B cell populations in a cohort of healthy Mexican individuals ranging from neonates to adults with the aim of establishing age-dependent reference values for distinct B cell populations in peripheral blood. We found that total memory and switched memory B cells increase throughout life and are maintained in adults, while transitional B cells decrease throughout life. These data may help to identify defects in peripheral B cell development in individuals suspected of suffering from PIDs. The results shown here demonstrate that important variations occur in B cell numbers and this should be taken into account when considering patients with potential immunological diseases.

## Materials and methods

### Study population

Blood samples from a total of 112 healthy subjects, taken between 2009 and 2014, were included in this study. Blood samples included 15 taken from umbilical cord, and 97 individuals ranging from birth to 40 years old. All blood samples were used for routine laboratory analysis of common haematological parameters. Immunological, infectious, and haemato-oncological diseases were ruled out in all individuals. Neonatal cord blood was obtained by venipuncture immediately after clamping. Samples were divided in the following groups: cord blood, new-borns, 1 month to 2 years, 3–5, 6–10, 11–18, and 19–40 years old. Demographic details of the tested population are presented in Table 1.

**Table 1** Ages and genders of the populations under study.

| Age group | Total number of subjects | Gender ratio F/M |
|---|---|---|
| Cord blood | 15 | 8/7 |
| 0–7 days | 6 | 2/4 |
| 1–24 months | 16 | 7/9 |
| 3–5 years | 19 | 6/13 |
| 6–10 years | 17 | 10/7 |
| 11–18 years | 18 | 8/10 |
| Adults | 21 | 13/8 |