ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
December 2022; 21(6):670-676.
Doi: 10.18502/ijaai.v21i6.11527

Biological Features of CD5+ CD19+ B1 Cell and Natural IgM (VH4-34) in Chronic Lymphocytic Leukemia vs. Multiple Sclerosis

Shaghayegh Pezeshki[1], Mir Hadi Jazayeri[1,2], Bahram Chahardouli[3], Nader Tajik[1,2], Seyed Massood Nabavi[4], and Mahdi Akbarzadeh[5]

[1] Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[2] Immunology Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
[3] Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Regenerative Medicine, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[5] School of Nursing, Baqiyatallah University of Medical Science, Tehran, Iran

Received: 6 August 2022; Received in revised form: 3 September 2022; Accepted: 14 September 2022

ABSTRACT

Chronic lymphocytic leukemia (CLL) is the clonal expansion of mature CD5+ B cells and the most common lymphoproliferative disease in adults (B1-CLL). B1 cells' anti-inflammatory effects include the production of natural IgM (nIgM) by the spleen and bone marrow, decreased inflammatory cytokines as a primary response to maintaining tissue homeostasis, and enhanced release of transforming growth factor β (TGFβ).

We used the flow cytometry technique in peripheral blood from patients with CLL and multiple sclerosis (MS) to immunophenotype B cells and their subpopulations. Whole blood from CLL and MS patients, as well as healthy controls, was used to detect nIgM using the VH4-34 gene copy number and real-time RT-PCR.

We found that the proportion of CD5+ B cells was significantly lower in MS patients than in the control group and that CD5+ B lymphocytes were significantly higher in CLL patients than in the control group. Compared to the control group, CLL patients had significantly higher levels of the VH4-34 gene copy number. On the contrary, MS patients had significantly lower VH4-34 gene copy number levels compared to the control group.

As the number of antibodies in CLL patients increases due to the high number of B1 cells, we propose a new way to treat MS by extracting this natural antibody from the sera of CLL patients and injecting it into MS patients.

Keywords: CD5+CD19+ B cells; Chronic lymphocytic leukemia; Multiple sclerosis; Natural IgM; VH4-34 gene

Corresponding Author: Mir Hadi Jazayeri, PhD; Department of Immunology, Faculty of Medical Sciences, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 912) 3541 067, Fax: (+98 21) 8857 7598, E-mail: hoohadi@yahoo.com

INTRODUCTION

The clonal expansion of mature CD5+ B cells is known as chronic lymphocytic leukemia (CLL), the most prevalent lymphoproliferative disease in adults.[1] Between 24% to 29% of all leukemias are CLL, typically affecting older people.[2] CD5- and CD5+ B cells are the two main subpopulations of B lymphocytes. Adults' peripheral blood lymphocytes, which comprise most peripheral blood B cells, contain about 10% CD5+ B cells. CD5+ B lymphocytes are a distinct B cell lineage from CD5-B cells that have unique functions and patterns of tissue distribution. These cells also express high levels of immunoglobulin (Ig) M and low levels of IgD.[3-5] CD5+ B cells are essential for innate immunity because they spontaneously express self-reactive natural antibodies in the absence of peripheral antigen stimulation [6] IgM isotypes, which can identify pathogens and self-antigens, make up the majority of these naturally occurring antibodies. They also play a role in the body's initial innate defense against pathogens. Natural IgM (nIgM) antibodies regulate the immune system against autoantigens and are essential for maintaining tissue homeostasis[7]. According to a prior study, cord blood samples contained the fewest autoantigen-specific antibodies, while older people had the highest levels of these antibodies.[8] The anti-inflammatory actions of B1 cells include the generation of nIgM by the spleen and bone marrow as a primary response to pathogen invasions, [9] the removal of necrotic and apoptotic cells,[10] the maintenance of tissue homeostasis [11] the reduction of inflammatory cytokines, and enhanced release of GcMAF.[12] Several VH genes encode natural antibodies, but the VH4-34 gene appears to be the most common[13]. As a member of the VH4 family, this gene has been extensively studied because the availability of this gene encodes MOAB (9G4) specific antibodies for immunoglobulin.[14] An interesting feature is that the framework region 1 (FWR1) mediates self-antigen binding, and the VH4-34 gene is essential for encoding nIgM.[15,16] Here, we assessed nIgM in patients with CLL and multiple sclerosis (MS) using the VH4-34 gene copy number.

MS is an autoimmune disease of the central nervous system. As mentioned above, the role of CD5+ B1 cells in the systemic inflammatory disorder has been evaluated and can open new research fields. Finding novel molecules produced by B1 cells to lessen and modulate the autoimmunity condition is a focus of nIgM research.[17]

It is shown in some studies that the therapeutic use of IgM in treating certain B-cell-mediated autoimmune diseases is clinically suitable because it can correct developmental defects.[6,9]

Additional studies on novel cellular interactions, such as B1 cell trafficking in various organs following autoimmune and inflammatory disorders, may raise several issues regarding the pathogenesis of inflammatory diseases. This study aimed to evaluate the frequency of CD5+ B1 lymphocytes in patients with CLL and compare it with CD5+ B1 cells in MS to determine whether CD5+ B1 cells or their byproducts (natural antibodies) can be used in MS treatment. Also, the possibility of using B1 cells or IgM for treating autoimmune diseases like MS was investigated since, to our knowledge, no study investigated the effect of immunomodulatory therapies on the B1 cell phenotype.

MATERIALS AND METHODS

Blood Samples

The study population comprised 30 patients with CLL (24 women and six men), aged 61 to 65 years, 30 patients with MS (26 women and four men), aged 39 to 45 years, and 30 healthy controls (25 women and five men), aged 56 to 58 years. After obtaining informed consent from the patients, between June 2019 and July 2020, fresh blood was collected from the study population by referring them to the Shariati Hospital. As a complementary test, patients suspected of CLL underwent flow cytometry and hematological examinations, and Patients suspected of MS underwent MRI. None of the patients had received chemotherapy or radiotherapy at least one year before and had not taken corticosteroids or immunosuppressive drugs. Exclusion criteria included any immune deficiency, autoimmune, systemic chemotherapy, systemic radiotherapy, infections such as tuberculosis, hepatitis C or B, and HIV.

Flow cytometry

100 µL of whole peripheral blood was added to the following flow cytometry tubes for immunostaining evaluations: (1) the tube containing 5 µL of anti-human CD5-PE (Becton-Dickinson, Germany), (2) the tube containing 5 µL of anti-human CD19-FITC (Becton-Dickinson, Germany) accounting for the

overlap of FITC and PE, and (3) the tube containing 5 µL of both monoclonal antibodies. In each experiment, monoclonal antibodies from the same IgG subclass served as the isotype control. The cells were stained and then incubated at 4°C for 30 min.

The cells were then washed with phosphate-buffered saline (PBS) for 5 minutes, centrifuged at 1500 rpm, and incubated in the dark at room temperature for 15 minutes. After incubation, the cell pellet was re-suspended in 1 mL of RBC lysis buffer. Becton Dickinson Caliber machine and Cell Quest Pro software were used for flow cytometry evaluations. A gate was first applied on the lymphoid population to filter out monocytes and granulocytes. Gating was performed until 100,000 cells were captured per gate. After gating, a quartile gate strategy was used for analysis on a correlated two-parameter screen (PE-anti-CD5 vs. FITC-anti-CD19).

VH4-34 Gene (Natural IgM) RT-PCR

The Easy-DNA Kit (Invitrogen Corp. Carlsbad, California, USA) was used to extract genomic DNA from whole blood (the B1 cells expressed a rearranged VH4-34 IgM). Gene rearrangements specific to VH4-34-D-JH were amplified using genomic DNA as a template. Using a primer designed to amplify the leader of VH4-34 (5′- AGATGTGAGTGTCTCAGGAATGC -3′ and 5′- TAACCACTGAAGGACCCACCATAGA -3′), 33, 100, and 300 ng of genomic DNA for each sample were amplified. The amplification process included a 2-minute initial denaturation step at 94°C, 20 to 35 cycles of 1 minute at 94°C, 1.5 minutes at 60°C, and 1.5 minutes at 72°C, and a 4-minute final extension step at 72°C. The amplified products were observed by electrophoresis through 2% agarose with 1 g/ml ethidium bromide.

Statistical Analysis

SPSS and PRISM software were recruited for statistical analysis (REST for quantitative real-time PCR data analysis). The ANOVA test was used to compare all the results and to look at the relationship between the data and the clinical characteristics. Statistical significance was defined as a p-value < 0.05.

RESULTS

Patients' Characteristics

Blood samples were collected from 30 MS patients, 30 CLL patients, and 30 healthy controls for this study. Table 1 lists the clinical and demographic data of each patient. According to the Binet system and the WHO criteria, the CLL stages are classified into stages A, B, and C. The MS stages (RRMS, SPMS, PPMS, and PRMS) were determined according to the McDonald's criteria.

Table1. The clinicopathological characteristics of CLL and MS patients

	CLL	MS
Number of Patients	30	30
Female	24	26
Male	6	4
Mean Age	53±2	49±3
Stage of Disease	Binet CLL Stage B	MS Stage of RRMS

CLL, chronic lymphocytic lymphoma; MS, multiple sclerosis

Comparison of CD5+CD19+ B Frequency in CLL and MS Patients and Controls

Based on the findings, the average percentage of peripheral lymphocytes that were CD19+ cells was 10%, ranging from 4 to 11%. In healthy peripheral blood, the proportion of CD19+CD5+ cells were 0.4 to 1.5% for lymphocytes and 5% to 37% for B cells (with the mean of 27%). Using the t-test, we examined CD5+CD19+ B cells in the peripheral blood of CLL and MS patients. The results showed that while CD5+CD19+ B lymphocytes are significantly higher in CLL patients than in the control group by a factor of 70.3, they are significantly lower in MS patients by a factor of 0.3 compared to the control group. However, sex, age, and disease stage had no significant correlation with B cell count. The detailed gating strategy is shown in Figure 1 and Table 2.

Evaluating Natural IgM in Patients with CLL and MS

Natural IgM was found in the whole blood of CLL and MS patients and one healthy control by the VH4-34 gene copy number. CLL patients had significantly higher levels of VH4-34 gene copy number than the control group. In comparison, MS patients had significantly lower levels of the VH4-34 gene copy number. Figures 2 represent the results.

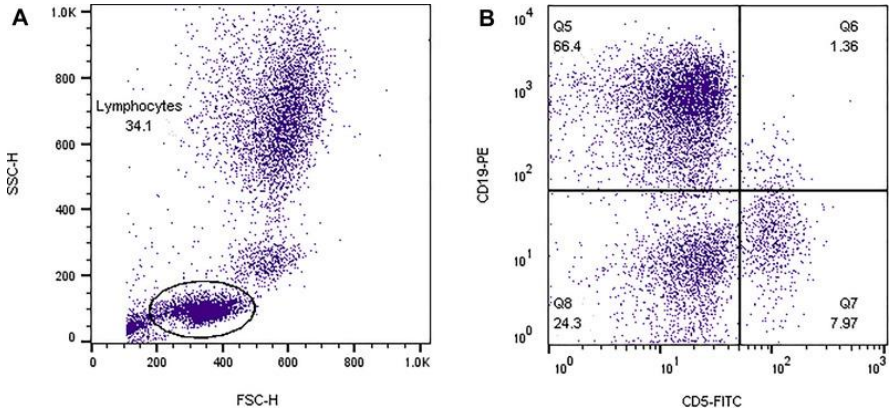

Figure. 1. Flow cytometry analysis of B1 cells as a representative of healthy control group. A) Gating of the lymphocyte on white blood cell (WBC) analysis based on side and forward scatter. B) CD5+CD19+ double positive staining A quadrant was drawn and double population of CD5 CD19 as Q6 are shown This data represents Normal groups.

Table 2. Intergroup comparison of B1 CD5 +CD19 + cell Frequency

Disease Group	Number	Mean	S.D	95% CI	Median	*p*
Normal	30	0.95	0.41	0.61, .81	0.71	<0.01
CLL	30	79.58	11.66	70.1, 3.2	73.5	
MS	30	0.62	0.32	0.41, .32	0.52	

CLL, chronic lymphocytic lymphoma; MS, multiple sclerosis; SD, standard deviation

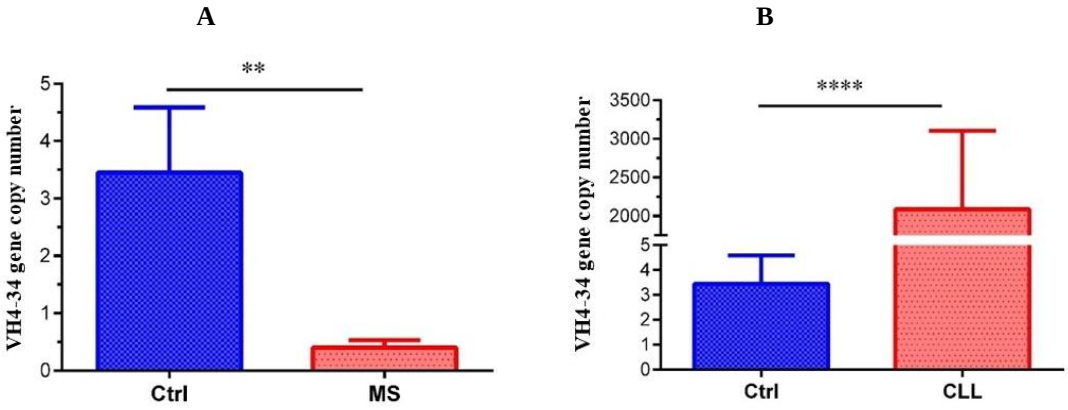

Figure 2. A) Comparison of VH4-34 gene copy number between healthy controls and MS groups (*p* value<0.009, fold change: 0.11) (Fold change<1). B) Comparison of VH4-34 gene copy number between CLL groups and healthy control (*p* value<0.0001, fold change: 605.60) ((Fold change≥1); CLL, chronic lymphocytic lymphoma; MS, multiple sclerosis, Ctrl, control group; RT-PCR, real-time polymerase chain reaction. ** *p*<0.01, ** *p*<0.0001**

DISCUSSION

We used flow cytometry to immunophenotype B cells and their subpopulations in the peripheral blood of patients with CLL and MS. We observed a significant decrease in the proportion of CD5+CD19+ B cells in MS patients compared to the control group and a significant increase in CD5+CD19+ B lymphocytes in CLL patients.

B1 cells are long-lived and self-renewing cells that are impartial to T cells.[18] B1 cells, as the main source of polyreactive auto-antibody, are critical for innate immunity and apoptotic clearance.[19] B1 cells are potential causes of autoimmune diseases like Sjogren's syndrome, SLE, and rheumatoid arthritis because they secrete autoantibodies. In fact, these patients have a higher-than-normal level of B1 cells in their peripheral blood.[20]

As an anti-inflammatory cytokine involved in regulatory T cell function, B cell differentiation, proliferation, and antibody production, B1 cells are the main source of IL-10. The impact of IL-10 on various autoimmune diseases (SLE vs. MS) is debatable.[21] Due to their ability to present antigens, produce cytokines and naturally occurring autoantibodies, B1 cells can exacerbate some conditions, such as SLE (B1 cells express B7-1 and B7-2).[22]

This study's results align with Hayakawa et al. They looked at CD5+CD19+ B lymphocytes in the peripheral blood of CLL patients and found a significant increase in the patient group.[23] Our findings are also consistent with Calvo et al. They examined CD5+ B1 cell levels in the peripheral blood of 111 CLL patients. They discovered significant increasedin CD5+ B1 cell levels between patients and controls.[24]

Compared to healthy control, Dorestwin. et al. found lower CD27+CD20+CD43+CD70- B1 cells in MS patients. Additionally, these results suggest the protective role of B1 cells in the Healthy Control group than MS.[25]

Some studies indicated the possible protective role of B1 cells in MS; Torring et al. reported a lower B1 cell count in MS patients, indicating an increase in remission in the MS group.[26]

Many studies have analyzed the function of CD5+CD19+ B1 cells in hepatic ischemia and cerebral),[27] intestinal, and other systemic inflammatory disorders, which may open novel research topics. (5) Regarding nIgM, numerous investigations have concentrated on finding novel molecules secreted by B1 cells to reduce and control the inflammatory state.[28]

According to some studies, mice with low levels of nIgM can produce IgG autoantibodies and are predisposed to developing severe MS. This is likely because the antigens and inflammation associated with apoptotic cells and debris can trigger B lymphocyte 2 (B2)cell reactions when not properly cleared.[29,30]

Studies have shown that polyclonal IgM treated IgM-deficient mice's developmental defects, suggesting that therapeutic use of IgM in the treatment of some B cell-mediated autoimmune diseases may have clinical relevance.[31-33]

Corticosteroids or immunosuppressive medications could not influence b cell reduction because neither of the patients in the current study had taken either of those drugs. Further analysis of lymphocyte populations using flow cytometry and immunohistochemistry seems necessary to determine the relationship between B cell circulating products.

The serum of healthy people contains natural autoantibodies, and with age, the level of these autoantibodies fluctuates. The total amount of serum IgG and IgM showed an increasing trend from cord blood samples up to samples from middle-aged people, while IgM showed a declining trend from the middle-aged to the elderly group).[8] Autoantibodies may exist before the onset of some diseases and are connected to the body's homeostasis. Natural antibodies against red blood cells commonly increase in hematologic diseases like anemia, polycythemia, leukocytosis, thrombocytopenia, and pancytopenia, autoantibodies, and may be useful as a biomarker Because CD5+CD19+ B lymphocytes have been shown to contribute to the spontaneous production of self-reactive nIgM in CLL and MS patients and healthy controls, we measured natural IgM detected by the VH4-34 gene copy number in whole blood from these patients as well as healthy controls. In contrast to the control group, CLL patients were found to have significantly higher levels of the VH4-34 gene copy number. Additionally, MS patients had significantly lower levels of the VH4-34 gene copy number than the control group, and this decline was found to be causal.

Small, mature-looking CD19+CD23+CD5+ B cells (B1 cell), which build up in the blood, bone marrow, and lymphoid organs, give clonal growth characteristics to

CLL. A natural antibody that protects against autoimmunity is also the most important byproduct of B1 cells. If the number of B1 cells in CLL patients increases the number of antibodies, we can develop a new method to treat autoimmune diseases. This natural antibody can be extracted and given to MS patients as an injection from the sera of CLL patients.

STATEMENT OF ETHICS

All procedures were carried out in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (No: IR.IUMS.FMD.REC.1397.3117), as well as with the local Ethics Committee of Iran University of Medical Sciences (IUMS).

FUNDING

This study was founded by the Iran University of Medical Sciences (IUMS-16047).

CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We acknowledge Iran university of medical sciences.

REFERENCES

1. Grywalska E, Zaborek M, Łyczba J, Hrynkiewicz R, Nied P. Chronic Lymphocytic Leukemia-Induced Humoral. Cells. 2020;9(11):2398.
2. Wall S, Woyach JA. Chronic Lymphocytic Leukemia and Other Lymphoproliferative Disorders. Clin Geriatr Med. 2016;32(1):175–89.
3. Bautista D, Vásquez C, Ayala-Ramírez P, Téllez-Sosa J, Godoy-Lozano E, Martínez-Barnetche J, et al. Differential Expression of IgM and IgD Discriminates Two Subpopulations of Human Circulating IgM+IgD+CD27+ B Cells That Differ Phenotypically, Functionally, and Genetically. Front Immunol. 2020;11:736.
4. Lundell A-C, Johansen S, Adlerberth I, Wold AE, Hesselmar B, Rudin A. High Proportion of CD5 + B Cells in Infants Predicts Development of Allergic Disease . J Immunol. 2014;193(2):510–8.
5. Sindhava VJ, Bondada S. Multiple regulatory mechanisms control B-1 B cell activation. Front Immunol. 2012;372.
6. Reyneveld GI, Savelkoul HFJ, Parmentier HK. Current Understanding of Natural Antibodies and Exploring the Possibilities of Modulation Using Veterinary Models . A Review. Front Immunol. 2020;11:2139.
7. Grönwall C, Silverman GJ. Natural IgM: Beneficial autoantibodies for the control of inflammatory and autoimmune disease. J Clin Immunol. 2014;34(SUPPL. 1):12–21.
8. Jazayeri MH, Pourfathollah AA, Rasaee MJ, Porpak Z, Jafari ME. The concentration of total serum IgG and IgM in sera of healthy individuals varies at different age intervals. Biomed Aging Pathol. 2013;3(4):241–5.
9. Rodriguez-Zhurbenko N, Quach TD, Hopkins TJ, Rothstein TL, Hernandez AM. Human B-1 cells and B-1 cell antibodies change with advancing age. Front Immunol. 2019;10(483):1–15.
10. Shao WH, Cohen PL. Disturbances of apoptotic cell clearance in systemic lupus erythematosus. Arthritis Res Ther. 2011;13(1):1–7.
11. Cautivo KM, Molofsky AB. Regulation of metabolic health and adipose tissue function by group 2 innate lymphoid cells. Eur J Immunol. 2016;46(6):1315–25.
12. Morris G, Puri BK, Olive L, Carvalho AF, Berk M, Maes M. Emerging role of innate B1 cells in the pathophysiology of autoimmune and neuroimmune diseases: Association with inflammation, oxidative and nitrosative stress and autoimmune responses. Pharmacol Res. 2019;148(25):104408.
13. Bhat NM, Adams CM, Chen Y, Bieber MM, Teng NNH. Identification of Cell Surface Straight Chain Poly-N-Acetyl-Lactosamine Bearing Protein Ligands for VH4-34-Encoded Natural IgM Antibodies. J Immunol. 2015;195(11):5178–88.
14. Locke IC, Leaker B, Cambridge G. A comparison of the characteristics of circulating anti-myeloperoxidase autoantibodies in vasculitis with those in non-vasculitic conditions. Clin Exp Immunol. 1999;115(2):369–76.
15. Pugh-Bernard AE, Silverman GJ, Cappione AJ, Villano ME, Ryan DH, Insel RA, et al. Regulation of inherently autoreactive VH4-34 B cells in the maintenance of human B cell tolerance. J Clin Invest. 2001;108(7):1061–70.
16. Bhat NM, Bieber MM, Spellerberg MB, Stevenson FK, Teng NNH. Recognition of auto- and exoantigens by V4-34 gene encoded antibodies. Scand J Immunol. 2000;51(2):134–40.
17. Mangge H, Prüller F, Schnedl W, Renner W, Almer G. Beyond macrophages and T cells: B cells and

immunoglobulins determine the fate of the atherosclerotic plaque. Int J Mol Sci. 2020;21(11):1–13.
18. Alugupalli KR, Leong JM, Woodland RT, Muramatsu M, Honjo T, Gerstein RM. B1b lymphocytes confer T cell-independent long-lasting immunity. Immunity. 2004;21(3):379–90.
19. Miles K, Simpson J, Brown S, Cowan G, Gray D, Gray M. Immune tolerance to apoptotic self is mediated primarily by regulatory B1a cells. Front Immunol. 2018;8(2):1–16.
20. Giltiay N V., Chappell CP, Clark EA. B-cell selection and the development of autoantibodies. Arthritis Res Ther. 2012;14(Suppl 4):1–13.
21. Ran Z, Yue-Bei L, Qiu-Ming Z, Huan Y. Regulatory B Cells and Its Role in Central Nervous System Inflammatory Demyelinating Diseases. Front Immunol. 2020;11(12):1–18.
22. Magatti M, Masserdotti A, Cargnoni A, Papait A, Stefani FR, Silini AR, et al. The role of b cells in pe pathophysiology: A potential target for perinatal cell-based therapy? Int J Mol Sci. 2021;22(7).
23. Hayakawa K, Formica AM, Brill-Dashoff J, Shinton SA, Ichikawa D, Zhou Y, et al. Early generated B1 B cells with restricted BCRs become chronic lymphocytic leukemia with continued c-Myc and low Bmf expression. J Exp Med. 2016;213(13):3007–23.
24. Calvo J, Places L, Espinosa G, Padilla O, Vilà JM, Villamor N, et al. Identification of a natural soluble form of human CD5. Tissue Antigens. 1999;54(2):128–37.
25. Rovituso DM, Duffy CE, Schroeter M, Kaiser CC, Kleinschnitz C, Bayas A, et al. The brain antigen-specific B cell response correlates with glatiramer acetate responsiveness in relapsing-remitting multiple sclerosis patients. Sci Rep. 2015;5(9):1–8.
26. Tørring C, Petersen CC, Bjerg L, Kofod-Olsen E, Petersen T, Höllsberg P. The B1-cell subpopulation is diminished in patients with relapsing-remitting multiple sclerosis. J Neuroimmunol. 2013;262(1–2):92–9.
27. Aziz M, Holodick NE, Rothstein TL, Wang P. The role of B-1 cells in inflammation. Immunol Res. 2015;63(1–3):153–66.
28. Zhang X. Regulatory functions of innate-like B cells. Cell Mol Immunol. 2013;10(2):113–21.
29. Vyshkina T, Kalman B. Autoantibodies and neurodegeneration in multiple sclerosis. Lab Investig. 2008;88(8):796–807.
30. Fetter T, Niebel D, Braegelmann C, Wenzel J. Skin-Associated B Cells in the Pathogenesis of Cutaneous Autoimmune Diseases-Implications for Therapeutic Approaches. Cells. 2020;9(12).
31. Roudafshani Z, Jazayeri MH, Mahmoudi AR, Nedaeinia R, Safari E, Jazayeri A. Evaluation of the frequency of CD5+ B cells as natural immunoglobulin M producers and circulating soluble CD5 in patients with bladder cancer. Mol Biol Rep. 2019;46(6):6405–11.
32. Justyna W. Natural IgM and the Development of B Cell-Mediated Autoimmune Diseases. Physiol Behav. 2017;176(5):139–48.
33. Darwiche W, Gubler B, Marolleau JP, Ghamlouch H. Chronic lymphocytic leukemia B-cell normal cellular counterpart: Clues from a functional perspective. Front Immunol. 2018;9:683.