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Regulatory T Cell Ratios in CD19 Deficiency Patients

CD19 Eksikliği Olan Hastalarda Regülatör T Hücre Oranları

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ÖZET

Amaç: CD19 eksikliği, "doğuştan gelen bağışıklık hataları (IEI)" sınıflandırmasında "baskın antikor eksiklikleri" grubunda yer alan yaygın bir değişken immün yetmezliktir (CVID). CD19 eksikliği, olan hastalarda otoimmün hastalıklar bildirilmiş olup bu hastalarda görülen otoimmün mekanizmalar hakkında literatürde veri bulunmamaktadır. Bu çalışmada CD19 eksikliği olan olgularda Foxp3 ekspresyonu ve düzenleyici T hücre (Treg)'lerin değerlendirilmesi amaçlandı.
Gereç ve Yöntem: Çalışmaya CD19 eksikliği tanılı üç hasta, genetiği doğrulanmış 11 taşıyıcı ve akraba olmayan 10 sağlıklı kontrol dahil edildi. Treg hücre oranları akım sitometrik olarak analiz edildi.
Bulgular: Hasta, taşıyıcı ve kontroldeki yardımcı T (Th) ve Treg hücresinin yüzdeleri ve ortalama floresan yoğunluğu (MFI) değerleri karşılaştırıldı. Th lenfosit yüzdeleri ve Treg belirteçlerinin yüzde değerleri açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu ($p>0,05$). Th yüzdeleri ve Treg yüzey belirteçlerinin yüzdesi açısından gruplar arasında istatistiksel olarak anlamlı fark bulunmazken ($p>0,05$), MFI açısından gruplar arasında istatistiksel olarak anlamlı bulundu ($p<0,05$).
Sonuç: CD19 eksikliği olan hastalarda Th ve Treg hücrelerde tespit edilen düşük MFI değerlerinin uygun olmayan T hücre aracılı immün yanıtlarla ilişkili olabileceğini düşündürdü. Ayrıca bu çalışma, CD19 eksikliğinde otoimmünite nedenini araştıran ilk çalışma özelliği taşımaktadır.

Anahtar Kelimeler: CD19, Foxp3, Treg

ABSTRACT

Aim: CD19 deficiency is a common variable immunodeficiency (CVID) that is in the group of "predominantly antibody deficiencies" in "inborn errors of immunity (IEI)" classification. Autoimmune diseases have been reported in patients with CD19 deficiency, and there is no data in the literature on autoimmune mechanisms seen in these patients. In this study, it was aimed to evaluate Foxp3 expression and regulatory T cell (Treg) markers in cases with CD19 deficiency.
Materials and Methods: Three patients diagnosed with CD19 deficiency, 11 genetically confirmed carriers and 10 unrelated healthy controls were included in the study. Treg cell markers were analyzed by flow cytometric analysis.
Results: Percentages and mean fluorescence intensity (MFI) values of helper T (Th) and Treg cell in patients, carriers and controls were compared. There was no statistically significant difference between the groups in terms of Th lymphocyte percentages and percentage values of Treg markers ($p>0.05$). Although there was no statistically significant difference between the groups in terms of Th percentages and percent of Treg surface markers ($p>0.05$), statistical significance was found between the groups in terms of MFI ($p<0.05$).
Conclusion: It suggested that low MFI values detected in Th and Treg cells in patients with CD19 deficiency may be associated with inappropriate T cell-mediated immune responses. In addition, this study is the first to investigate the cause of autoimmunity in CD19 deficiency.

Key words: CD19, Foxp3, Treg

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INTRODUCTION

CD19 deficiency is a common variable immunodeficiency (CVID) that is in the group of "predominantly antibody deficiencies" in "inborn errors of immunity" classification and was first identified in 2006 by our group in four cases.1-3 To date, only 11 cases of CD19 deficiency have been reported in the literature. In CD19 deficiency, there is a predisposition to autoimmune diseases as well as recurrent infections.2,4,5 However, the reasons for this predisposition have not yet been fully elucidated. Regulatory T cells (Treg) are cells that maintain immune tolerance and function in the prevention of autoimmune diseases and are characterized by the expression of CD4, CD25 and fork head box P3 (FoxP3)6,7 and the cells secrete suppressor cytokines interleukin (IL)-10 and transforming growth factor-beta (TGF-β)1.8 Foxp3, a member of the Fox protein family and functioning as a transcription factor, plays a role as a master regulator in the development and function of Treg cells.9,10

Functional dysfunction of Treg cells may result in inadequate regulation of immune responses and autoimmune diseases.10 There are no studies on Treg cell in CD19 deficiency where autoimmune diseases can be seen.

In this study, it was aimed to evaluate the surface markers of Treg cell and intracytoplasmic Foxp3 expression in the peripheral blood of individuals with defined CD19 deficiency.

MATERIAL AND METHODS

Patients

The study was performed in the Pediatric Immunology and Allergy Department in January 2023. Three patients diagnosed with CD19 deficiency (c.973_973insA; p.Arg325AlafsX4; GenBank: AH005421.2),3,11-13 11 individuals known to be heterozygous (carrier) genotype for the relevant mutation, and 10 healthy controls were included into the study. The studies reported herein were approved by Institutional Review Board (protocol number 2020/2383). Written informed consent was obtained from participants. Healthy controls were shown not to be patients or carriers in terms of CD19 deficiency by flow cytometry. Two ml blood samples were taken from all patients, carriers and healthy controls into K3-EDTA tubes for flow cytometry analysis.

Flow Cytometry Analysis

Treg cells were evaluated as flow cytometric in peripheral blood samples taken from patients, carriers and healthy controls. For this purpose, surface and intracellular staining was performed using CD25 (PE Cy7-eBioscience, Frankfurt, Germany), CD127 (AlexaFlour 647-eBioscience, Frankfurt, Germany), CD4 (FITC-eBioscience, Frankfurt, Germany) ve FoxP3 (PE-eBioscience, Frankfurt, Germany) monoclonal antibodies (mAb). After staining, cell count was performed with a Becton Dickinson Canto II (BD Biosciences, Heidelberg, Germany) flow cytometer device and (at least 10x103 cells from each patient and controls) was analyzed with the FACS Diva software version 6.1.3. program (Figure 1). As a result of the analysis, the percentages and mean fluorescence intensity (MFI) values of CD4+ CD25+ CD127-, CD4+ FoxP3 and CD4+ CD25+ cells were determined.

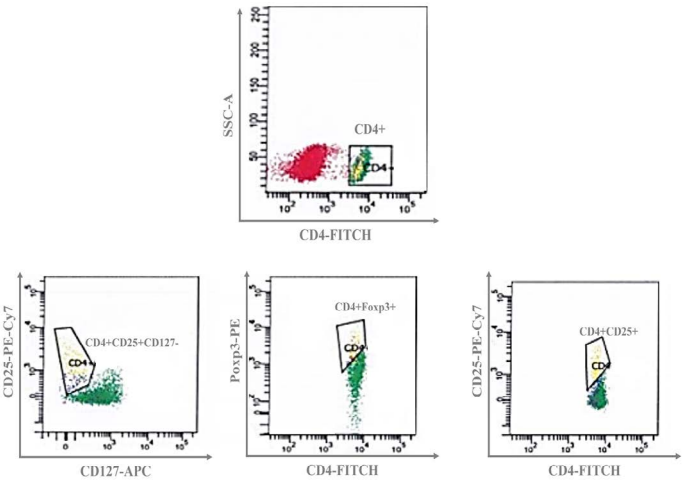

Figure 1. Flow cytometric analysis of Foxp3 expression

Statistical Analysis

Kruskal-Wallis Test was used for statistical data analysis, and variant test analysis Post-hoc Bonferroni corrected Mann-Whitney U Test was used to compare parameters between groups. $p<0.05$ was considered statistically significant. Statistical analyzes were performed using the SPSS program (SPSS for windows, USA).

RESULTS

The M/F ratio of the patient group was 1/2, and the mean age was 21.6±10.2 years. The M/F ratio of the carrier group was 6/5, the mean age was 33.2±27.5 years, and the M/F ratio of the control group was 6/6, the mean age was 36.9±4 years. There was no statistically significant difference between the groups in terms of age and gender ($p>0.05$).

The patient, carrier and control groups were compared in terms of the percentages and MFI of CD4+ Th lymphocyte and Treg cell. The percentages of CD4+ Th lymphocyte (33.7±16.01 in the patient group; 31.3±11.7 in the carrier group; 29.6±9.3 in the control group), CD4+ CD25+ CD127- T cell (5.9±2, 2; 5.6±1.5; 6.6±1.7), CD4+ FoxP3+ cells (6.6±1.1; 4.6±1.6; 5.8±2.1) and CD4+CD25+ T cells (7.8±2.1; 6.8±2.4; 6.5±2.1) were similar and no statistically significant results were found in the percentages of these T cells ($p>0.05$). On the other hand, when evaluated in terms of MFI values, statistical significance was found between the groups (Table 1,

Table 1. Mean fluorescence intensity values of Th and Treg cells of the patient, carrier and control groups

Cells	Patients (n=3) (Mean±SD)	Carriers (n=11) (Mean±SD)	Control (n=10) (Mean± SD)	P value
CD4+	2697±721	2798±1060	5100±1118	
	1871-3204	2491-3106	3538-6465	p=0.001
CD4+CD25+CD127-	2280±683	2493±122	4370±947	
	1508-2807	2283-2654	3061-5685	p=0.001
CD4+ Foxp3	2565±846	2728±231	4458±837	
	1589-3103	2481-3183	3122-5394	p=0.001
CD4+ CD25+	2508±725	2614±169	4526±969	
	1725-3156	2411-2938	3226-5617	p=0.001

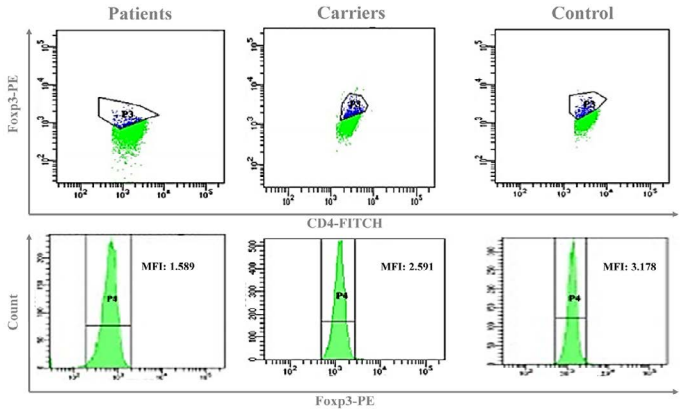

Figure 2. Foxp3 MFI values of patients, controls and carriers

Figure 2).

DISCUSSION

CD19 deficiency is a rare, predominantly antibody deficiency. Although frequent infection is a common condition in CD19 deficiency, it can also be seen also autoimmune diseases.4,5 However, there is no study in the literature on the mechanism of autoimmune diseases seen (developed/occurred could be better) in CD19 deficiency. In this study, in which the surface and intracellular markers of Treg cell in the peripheral blood of patients with CD19 deficiency and carriers and compared with the control group. The MFI values of Th and Treg cell were found to be lower than the healthy control. These findings suggested that Th cells might be affected in CD19 deficiency and T cell-mediated immune dysregulation might be related with autoimmunity.

Th cells have an important role in directing immune responses and are usually numerically and functionally affected in IEI. Although there was no statistical difference in terms of the percentages of T cells (CD4 Th cells) in this study between the groups, MFI values were found to be statistically significant ($p<0.05$). The MFI value shows the concentration of the relevant molecule on the cell surface or cell cytoplasm. The MFI value is often used in flow cytometric analyzes to compare the expression of the target of interest between cell populations and may be directly related to the function of the cell.14 The low MFI value of Th cells in CD19 deficiency may be related to the functional insufficiency of Th cells.15 These findings are similar to the findings of a study that showed that impaired responses may develop after Th lymphocyte activation in CD19 deficiency.15 In the light of these findings, T cell-mediated immune tolerance may be impaired in these patients, and after impaired T lymphocyte immune tolerance, antibody production of B lymphocytes might also be affected, resulting in antibody responses to our own antigens.

Treg cells, a subgroup of Th cells, play an important role in maintaining tolerance to our own antigens and alloantigen unresponsiveness.6,16,17 FoxP3 is a crucial transcription factor required to induce and stabilize the specific phenotype and functional properties of Treg cells.18 Defects in FoxP3 expressing Treg cell development and function can lead to many immunological disease.7 Patients with IEI may have various viral and bacterial infections, as well as lymphoproliferation, cancer and autoimmune diseases, and close follow-up of these patients is important as they may occur during the follow-up of the patients.9 In this study, the MFI values of Treg cell markers were evaluated in CD19 deficiency and were found to be significantly lower when compared to the control group ($p=0.001$). The low levels of both cell surface markers (CD4, CD25 and CD127) and intracellular marker (Foxp3) of Treg cells in the carrier and patient groups suggested that a T cell-mediated immune dysregulation might be and related with autoimmunity. Systemic lupus erythematosus and rheumatoid arthritis-like joint inflammations reported in a proportion of CD19 deficient patients might be explained by impaired Treg cell function.19 In addition, decreased Foxp3 MFI value may be associated with functionally reduced immune tolerance and subsequent autoimmune diseases. Detection of these findings in CD19 deficiency might indicate that the function of Treg cells is affected and impaired T cell-mediated immune tolerance.

The intracytoplasmic Foxp3 expression of T cell correlates with the number of Treg cell determined by extracellular

staining as the CD4+ CD25+ CD127- subpopulation. This confirms the correlation between simultaneous high expression of CD25 molecule and low expression of CD127 molecule and expression of intracellular factor Foxp3.20,21 In our study, it was seen that the surface markers used to identify the percentages of Treg and the intracellular Foxp3 marker were compatible with each other in all our blood samples (patient, carrier, control). Thus, we though that our flow cell meter study has complete reliability in itself.

Treg cells carry out their duties in autoimmunity in the immune system, not alone, but by acting together with other Th cell subgroups.22 For example, IL-4 secreted by Th2 cells and IL-17 secreted by Th17 cells negatively affect Treg cell functions.23,24 In another study, it was reported that the problem that will occur in Th2 cell groups negatively affects Treg cells and causes the development of autoimmune diseases.25-26 The low MFI of molecules in Th cells and Treg cells detected in our study seems to be compatible with the information in the literature.

The limitation of this study is that this is not a functional study as we evaluated the markers of Th and Treg cell using surface and intracytoplasmic staining. We thought that more enlightening data would be obtained on the status of helper T lymphocyte and Treg-related immune dysregulation with further functional studies in CD19 deficiency.

In conclusion, this is the first study to evaluate the markers of Treg cell in CD19 deficiency. We believe that the low MFI value of Th lymphocyte and Treg cell that we detected in our CD19-deficient patients and carriers may be related to T cell-mediated immune dysregulation that may develop in these patients.

Etik Kurul: The studies reported herein were approved by Institutional Review Board (protocol number 2020/2383).

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