B cells and T cells Abnormalities in Patients with Selective IgA Deficiency

Yasser Bagheri[1], Tannaz Moeini Shad[2], shideh namazi[3], Gholamreza Azizi[4], Ali Hosseini[5], mohsen saeidi[1], farzaneh tofighi[6], Fereshte Salami [7], salar Pashangzadeh [8], Samaneh Delavari[2], babak mirmanachi[9], Nima Rezaei[10], Hassan Abolhassani[2], Asghar Aghamohammadi[11], and Reza Yazdani[12]

[1]Golestan University of Medical Sciences
[2]Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science
[3]Iran University of Medical Sciences
[4]Non-Communicable Diseases Research Center, Alborz University of Medical Sciences
[5]Golestan University of Medical Sciences and Health Services
[6]Tehran University of Medical Sciences
[7]Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Science, Tehran, Iran
[8]Iranian Primary Immunodeficiencies Network (IPIN), Tehran University of Medical Sciences, Tehran, Iran
[9]Duke University Division of Gastroenterology
[10]Tehran University of Medical Sciences Childrens Hospital
[11]RCID
[12]Research Center for Immunodeficiencies

January 18, 2021

Abstract

Background: Selective IgA deficiency (SIgAD) is the most prevalent primary immunodeficiency with almost unknown etiology. This study aimed to investigate the clinical diagnostic and prognostic values of lymphocytes subsets and function in symptomatic SIgAD patients. Methods: A total of 30 available SIgAD patients from the Iranian registry and 30 age-sex-matched healthy controls were included in the present study. We analyzed B and T cell peripheral subsets and T cell proliferation assay by flow cytometry in SIgAD patients with mild and severe clinical phenotypes. Results: Our results indicated a significant increase in naïve and transitional B cells and a strong decrease in marginal zone-like and switched memory B-cells in SIgAD patients. We found that naïve and central memory CD4+ T cell subsets, as well as Th1, Th2 and regulatory T cells have significantly decreased. On the other hand, there was a significant reduction in central and effector memory CD8+ T cell subsets, whereas proportions of both (CD4+ and CD8+) terminally differentiated effector memory T cells (TEMRA) were significantly elevated in our patients. Although some of T cell subsets in severe SIgAD were similar, decrease in marginal-zone and switched memory B cells and increase in CD21low B cell of severe SIgAD patients were slightly prominent. Moreover, the proliferation activity of CD4+ T cells was strongly impaired in SIgAD patients with a severe phenotype. Conclusion: SIgAD patients have varied cellular and humoral deficiencies. Therefore, T cell and B cell assessment might help in better understanding the heterogeneous pathogenesis and prognosis estimation of the disease. Keywords: Primary immunodeficiency, Selective IgA deficiency, B cell subsets, T cell subsets, flow cytometry, proliferation assay

B cells and T cells Abnormalities in Patients with Selective IgA Deficiency

Yasser Bagheri[1], Tannaz Moeini Shad[2], Shideh Namazi[3], Gholamreza Azizi[4], Ali Hosseini[1], Mohsen Saeidi[5], Farzaneh Tofighi Zavareh[2,6], Fereshteh Salami[2], Salar Pashangzadeh[2], Samaneh Delavari[2], Babak Mirminachi[7], Nima Rezaei[2,8,9], Hassan Abolhassani[2,10], Asghar Aghamohammadi[2*], Reza Yazdani[2,11*]

1. *Clinical Research Development Unit (CRDU), 5 Azar Hospital, Golestan University of Medical Sciences, Gorgan, Iran*
2. *Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
4. Non-*Communicable Diseases Research* Center, Alborz University of Medical Sciences, Karaj, Iran
5. *Stem cell research center, Golestan university of medical sciences, Gorgan, Iran*
6. *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
7. *Department of Medicine, Division of Gastroenterology, Duke University, Durham, NC, USA*
8. *Department of Immunology, School of Medicine, Tehran University of Medical Science,Tehran, Iran.*
9. *Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran*
10. *Division of Clinical Immunology, Department of Laboratory Medicine, Karolinska Institute at Karolinska University Hospital Huddinge, Stockholm, Sweden*
11. *Primary Immunodeficiency Diseases Network (PIDNet), Universal Scientific Education and Research Network (USERN), Tehran, Iran*

*** Corresponding author:** Asghar Aghamohammadi, MD, PhD.

Address: Children's Medical Center Hospital, 62 Qarib St., Keshavarz Blvd., Tehran 14194, Iran.

Tel: + 98 21 6642 8998

Fax: + 98 21 6692 3054

Email:aghamohammadi@sina.tums.ac.ir

*** Corresponding author:** Reza Yazdani, PhD.

Address: Children's Medical Center Hospital, 62 Qarib St., Keshavarz Blvd., Tehran 14194, Iran

Tel: + 98 21 6642 8998

Fax: + 98 21 6692 3054

E-mail: reza_yazdani86@yahoo.com

Abstract

*Background***:** Selective IgA deficiency (SIgAD) is the most prevalent primary immunodeficiency with almost unknown etiology. This study aimed to investigate the clinical diagnostic and prognostic values of lymphocytes subsets and function in symptomatic SIgAD patients.

*Methods***:** A total of 30 available SIgAD patients from the Iranian registry and 30 age-sex-matched healthy controls were included in the present study. We analyzed B and T cell peripheral subsets and T cell proliferation assay by flow cytometry in SIgAD patients with mild and severe clinical phenotypes.

*Result***s:** Our results indicated a significant increase in naïve and transitional B cells and a strong decrease in marginal zone-like and switched memory B-cells in SIgAD patients. We found that naïve and central memory $CD4^+$ T cell subsets, as well as Th1, Th2 and regulatory T cells have significantly decreased. On the other hand, there was a significant reduction in central and effector memory $CD8^+$ T cell subsets, whereas proportions of both ($CD4^+$ and $CD8^+$) terminally differentiated effector memory T cells (T_{EMRA})

were significantly elevated in our patients. Although some of T cell subsets in severe SIgAD were similar, decrease in marginal-zone and switched memory B cells and increase in $CD21^{low}$ B cell of severe SIgAD patients were slightly prominent. Moreover, the proliferation activity of $CD4^+$ T cells was strongly impaired in SIgAD patients with a severe phenotype.

Conclusion: SIgAD patients have varied cellular and humoral deficiencies. Therefore, T cell and B cell assessment might help in better understanding the heterogeneous pathogenesis and prognosis estimation of the disease.

Keywords: Primary immunodeficiency, Selective IgA deficiency, B cell subsets, T cell subsets, flow cytometry, proliferation assay

Key message: We analyzed B and T cell peripheral subsets and T cell proliferation assay by flow cytometry in SIgAD patients. Our results indicated significant abnormalities in B cell patterns similar to CVID patients. Based on phenotype analyses, we observed some more abnormalities in SIgAD patients with severe phenotypes such as a high subpopulation of CD21low B cells and T cell proliferation defect. Accordingly, severe patients manifest a higher number of respiratory infections compared to mild SIgAD, suggesting further follow up and more precise management in these patients. The findings of the present study suggest that the investigation of B and T cell subsets could be helpful for a better understanding of the pathogenesis and prognosis of the disease.

Introduction

Selective IgA deficiency (SIgAD) is the most prevalent primary immunodeficiency disorder (PID), identified by serum concentration of IgA below 7 mg/dL and normal concentrations of IgG and IgM in patients over 4 years of age. The majority of SIgAD patients are asymptomatic, although some of them manifest different clinical manifestations, including gastrointestinal and respiratory tract infections, allergic diseases and autoimmune disorders. The disease progression to CVID in a selected group of SIgAD patients with IgG subclasses deficiency or autoimmune disorders has been reported (1).

Besides monogenic defects defined in a minority of SIgAD patients (2), no specific causes for the pathogenesis of disease have not been reported. However, defects in the process of IgA class switch recombination (CSR), IgA production and secretion, as well as the long-term survival of IgA, switched memory B cells and plasma cells of SIgAD patients have been identified in unsolved cases (3). Defects in these immunologic processes are associated with abnormalities in the lymphocytes of SIgAD patients. Hence, the assessment of the lymphocytes, especially B cell and T cell subsets, could be valuable and helpful. Several studies have demonstrated B cell and T cell abnormalities in some groups of SIgAD patients (4, 5). Regarding B cell subsets, a decrease in the number of switched memory B cells, IgA plasma cells and transitional $IL\text{-}10^+$ regulatory B cells of SIgAD patients has been reported (6-9). On the other hand, defect in some T cell subsets of SIgAD cases has been reported that is linked to insufficient IgA-producing B cells (6, 9, 10).

Flow cytometric immunophenotyping can play an important role in the diagnosis, prognosis, classification and management of patients with SIgAD. Hence, for the first time, we aimed to investigate the main subpopulations of B and T lymphocytes along with an evaluation of T cell function, to clarify the correlation between immunological characteristics and clinical manifestations in symptomatic patients with SIgAD.

Material and Methods

Patients

A total of 30 available symptomatic SIgAD patients (from the Iranian PID registry (11)) and 30 age-sex-matched healthy controls (HCs) were included in the present study. The patients had referred to the Children's Medical Center (Pediatrics Center of Excellence affiliated to Tehran University of medical sciences, Tehran, Iran). All patients were diagnosed with SIgAD according to the European Society for Immunodeficiencies (12). The study was approved by the Ethics Committee of Tehran University of Medical and written informed consent was obtained from all the individuals (IR.TUMS.VCR.REC.1396.2018). Demogra-

phic, clinical manifestations and immunological data of the patients were documented in a questionnaire form.

Classification of patients

To compare demographic, clinical and immunological data, the patients were categorized into two groups severe and mild (based on clinical manifestations) as well as consanguine and non-consanguine groups (based on parental consanguinity). The patients were divided into two groups of mild and severe, as patients with, severe infections (e.g. bloodstream, central nervous system, and deep-seated infections like osteomyelitis and arthritis), autoimmunity, or malignancy were categorized in the severe group and others were considered as a mild group

Lymphocyte subsets assay

Peripheral blood mononuclear cells (PBMCs) were separated from blood samples collected in sodium heparin tubes using a Ficoll-Hypaque density gradient centrifugation, at 600g for 25 minutes at 22@C. For extracellular staining, PBMCs were split into 5-panel fractions and stained for 20 min at 2-8 ^{o}C in the dark place with each cocktail containing the monoclonal antibodies at optimal concentrations. The B1 panel was utilized to determine naïve ($CD19^{+}$, $CD27^{-}$, IgM^{+}, IgD^{+}), IgM only memory ($CD19^{+}$, $CD27^{+}$, IgM^{++}, IgD^{-}), switched memory ($CD19^{+}$, $CD27^{+}$, IgM^{-}, IgD^{-}) and marginal zone-like B cells ($CD19^{+}$, $CD27^{+}$, IgM^{++}, IgD^{+}). The B2 panel was used to identify $CD21^{low}$ B cells ($CD19^{+}$, $CD21^{-/low}$,$CD38^{-/low}$, IgM^{+++}), plasmablast ($CD19^{+}$, $CD21^{-/low}$, $CD38^{++/+++}$, IgM^{-}) and transitional B cells ($CD19^{+}$, $CD21^{+}$, $CD38^{++}$, IgM^{+}). The T1 panel was used to classify naïve ($CD4^{+}$ or $CD8^{+}$, $CD45RA^{+}$, $CCR7^{+}$), effector memory ($CD4^{+}$ or $CD8^{+}$, $CD45RA^{-}$, $CCR7^{-}$), central memory ($CD4^{+}$ or $CD8^{+}$, $CD45RA^{-}$, $CCR7^{+}$) and T_{EMRA} (terminally differentiated effector memory) T cells ($CD4^{+}$ or $CD8^{+}$, $CD45RA^{+}$, $CCR7^{-}$). The T2 panel was used to classify regulatory T cells (Tregs, $CD4^{+}$, $CD25^{+}$, $FOXP3^{+}$, $CD127^{-/low}$). The information about panels is provided in **Table S1** . For intracellular staining, following the surface molecule staining, they were fixed and permeabilized throughout FOXP3/Permeabilization buffer (eBioscience, US) according to manufacturer's instructions for the following: Anti-Human FOXP3 (PE), Anti-Human IL-17 (PE), Anti-Human IL-4 (APC) and Anti-Human IFN-γ (FITC). All antibodies and isotype controls were purchased from eBioscience corporations.

To assess T helper cells (including Th1, Th2 and Th17), 1×10^{6} (cell/mL) of PBMCs were cultured within in the Roswell Park Memorial Institute (RPMI 1640) cell culture medium, followed by stimulation with phorbol myristate acetate (PMA, 50 ng/mL, Sigma-Aldrich, US) / ionomycin (1μg/mL, Sigma-Aldrich), and in the presence of brefeldin (5μg/mL, eBioscience). Then, the cells were incubated at 37 ^{o}C in 5% CO2 and 95% humidity incubator for 5 hours. The stimulated cells were washed with phosphate-buffered saline (PBS), and surface staining with Anti-Human CD4 (PerCp)-Cy5.5 was performed. The gating strategy is similar to our previous study (13).

T cell proliferation assay

To assess T cell proliferation, PBMCs were cultured with fluorescent 5,6-carboxyfluorescein succinimidyl ester (CFSE, Biolegend, US). A CFSE stock solution was prepared by dissolving CFSE in dimethyl sulfoxide (DMSO) at a concentration of 5 mM/L, based on the manufacturer's instructions. This stock was frozen in small aliquots to prevent excessive freeze and thaw cycles. CFSE was diluted with PBS in a ratio of 1:501 and it was added to a transverse falcon tube containing 500μL of PBMC suspension (5×10^{6} cell/mL) in RPMI 1640 cell culture medium containing 10% FBS (fetal bovine serum, Biosera, France). The tube turned rapidly and vortexed to ensure homogenous dispersal. After labeling, the cell suspension was incubated for 5 minutes at 37^{o}C. Then, 9 mL of RPMI1640 containing 10% FBS was added to the cell suspension and it was centrifuged at 500 g for 5 minutes. Cells were washed three times and 1mL of RPMI1640 with 10% FBS was added. Regarding T cell stimulation and proliferation, anti-CD3 antibody (1 μg/ml) was added to 500 μL of sterile PBS, and the plate was incubated at 37@C for 2 hours. The coated plate was washed twice with sterile PBS and the labeled cells were directly added, and finally, anti-CD28 antibody (2 μg/ml) was added as a stimulant for T cells. The plate was incubated at 37 ^{o}C in 5% CO_2 and 95% humidity incubator for 96

hours. An unstimulated well was considered as a control for non-proliferative cells. After 96 hours, the cells were harvested and washed. After staining with Anti-Human CD4 (PerCPCy5.5), the cells were eventually analyzed by BD FACSCalibour^{TM}Flocytometer and CellQuest Pro software (BD, Biosciences, San Jose, CA, USA). Proliferation analysis was performed by comparing three criteria, including the percentage of cells divided (%divided), the average number of cell divisions that a cell undergoes (Division Index), and the average number of cell divisions that occur in the entire primary cell population (Proliferation Index) by FlowJo 7.6 software.

Statistical analysis

SPSS software (Windows version 16.0; SPSS Inc., Chicago, IL, USA) was utilized for statistical analysis. We used the Kolmogorov-Smirnov test to estimate whether data were normally distributed. The findings were presented as median (interquartile range [IQR], presented as a range with 25th–75th percentiles) values. A chi-squared test or Fisher's exact test was utilized for comparisons. The differences were considered statistically significant for P -values that were < 0.05.

Results

Demographic, immunologic and clinical findings

Among all registered Iranian SIgAD patients, 30 available patients (23 males and 7 females) were recruited in the present study. The median (IQR) age of patients was 11 (7.3-16) years at the time of the study. Parental consanguinity was present in 16 (53.3%) of patients. Pneumonia (42.3%) was the most frequent clinical manifestation in the studied patients and malignancy was not diagnosed in this cohort. The demographic, clinical and immunologic characteristics of patients are summarized in **Table S2** . After categorizing patients based on severe and mild phenotypes, demographic, clinical and immunologic data were compared (**Table 1**). The patients with severe phenotype manifested more respiratory complications compared with the mild group. Other parameters have not demonstrated a significant difference. Also, the patients with severe phenotype had significantly higher serum IgM level (p =0.03), demonstrating a super-switch in switching to IgM in severe patients. The same comparisons were performed in patients with and without consanguinity, which none of them had a significant difference (**Table S3**). In contrast, patients with mild phenotype presented a slightly higher rate of allergy and gastrointestinal manifestations with an increased serum IgE level, suggesting a shift from IgA switching toward IgE production (**Table S2**).

B-cell subsets

SIgAD patients showed significantly an increased frequency of CD19^{+} B-cells [11.2 % (9.4-13.07%) *vs.*7.2% (6-8.6%), p <0 .0001], with increased naïve B-cells [71% (63.7-80%) *vs.* 66.5% (56.2-71.1%),p =0.036] and transitional B-cells [8% (3.6-13.5%)*vs.* 4.8% (2.6-9.5%), p =0.032] compared with HCs. In contrast, the percentage of marginal zone-like [2.3% (2-3.5%)*vs.* 3.4% (2.3-4.8%), p =0.022) and switched memory B-cells [3.5% (1.9-5.5%) vs. 6% (3.5-8.4%), p =0.006] were significantly lower than HCs. However, decreased IgM-only memory and plasmablasts and increased CD21^{low} B-cells in patients than those in HCs were not significant **(Table 2** and**Figure 1).** Interestingly, some comparisons were significant between patients' clinical groups. The percentage of CD19+ B cells in both mild and severe phenotypes was significantly higher than HCs [11.6% (8.7-13) *vs.* 7.2% (6-8.6), p =0.000, 10.8% (9.6-13.2) *vs.* 7.2% (6-8.6), p =0.000], respectively. Severe SIgAD patients demonstrated a significant increase in the percentage of CD21^{low} B-cells [1.5% (1-2.2)*vs.* 2.7% (1.6-5.6), p =0.025], and a significant decrease in the percentage of both marginal-zone and switched memory B cell subsets [3.4% (2.3-4.8) *vs.* 2.2% (2-3), p =0.040, 6% (3.5-8.4) *vs.* 2.7% (1.7-4.7), p =0.003], respectively. The percentage of transitional B cells in mild SIgAD patients was higher than HCs [10.7% (3.9-13.8) *vs.* 4.8% (2.6-9.5), p =0.047] **(Table 3** and **Figure 2)** . The percentage of IgM-only memory was significantly higher in patients with consanguinity than those without consanguinity (**Table S4**). We also categorized the frequency of B cell subsets of SIgAD patients into three categories: normal, decreased and increased based on the normal range of HCs (**Table S5**). Based on this analysis, the most decrease in B cell subsets is related to switched memory B-cells (23%), while the most increase is related to naïve B cells (27%) in SIgAD patients. Except for increased CD21^{low} B-cells in severe SIgAD patients compared with

mild SIgAD patients, none of the rest B-cell subsets showed a significant link with clinical severity (**Table S6**).

T-cell subsets

The subset separation of $CD4^+$ T cells revealed a significant reduction in total $CD4^+$ T cells [36.5% (30.8-41.2%) *vs.* 40.1% (37.3-47.2%), p =0.038)], central memory cells [11% (7.3-13.1%) *vs.* 24.5% (16-29%),p <0.0001), Th1 [7.7% (5.2-9.9%) *vs.* 12% (7.8-16%), p =0.002], Th2 [0.3% (0.2-0.4%) *vs.* 0.6% (0.4-1.3%), p <0.0001] and Tregs [0.3% (0.03-0.7%) *vs.* 1.4% (1.1-1.6%), p <0.0001] in patients compared with HCs. Oppositely, the percentage of T_{EMRA} [9.5% (5.7-16.4%) *vs.* 2% (1.3-6.2%),p <0.0001] was meaningfully higher than HCs. Moreover, decreased effector memory and Th17, and increased naïve helper T cells in patients in comparison with HCs were not significant (**Table 2** and **Figure 1**). Regarding the percentage of $CD8^+$ T cell subsets, central memory [0.6% (0.3-0.8%) *vs.* 3% (2-6%), p <0.0001] and effector memory [12.3% (7.6-22.1%) *vs.* 23.9% (19.5-27.2%),p <0.0001] were markedly diminished in patients compared with HCs. On the other hand, the percentage of cytotoxic T_{EMRA} [44.1% (28.4-55.7%) *vs.* 24.4% (20-31%), p <0.0001] was significantly higher than HCs. However, increased total $CD8^+$ T cells and decreased naïve $CD8^+$ T cells were not significant in patients compared to those in HCs (**Table 2** and **Figure 1**).

Regarding the comparison of the percentage of $CD8^+$ T cells subsets between severe SIgAD patients with HCs, naïve T cells, effector and central memory cells demonstrated a significant reduction. Also, there was a significant decrease in the percentage of total $CD4^+$ T-cells, central memory, Th1, Th2, and regulatory T cells, whereas T_{EMRA} in both $CD4^+$and $CD8^+$ T cells demonstrated an increase. On the other hand, the percentage of effector and central memory cells within $CD8^+$ T cells, as well as central memory, T_{EMRA}, Th1 and regulatory T cells within $CD4^+$ T cell subsets demonstrated a significant decrease in mild forms of SIgAD compared to HCs. In contrast, we found an increase in T_{EMRA} $CD8^+$ T cells and Th2 $CD4^+$ T cells in mild patients compared to controls (**Table 3** and **Figure 2**). Comparisons of the percentages of all T cell subsets between SIgAD patients with and without consanguinity have not indicated any significant difference (**Table S7**). We also categorized the frequency of T cell subsets of SIgAD patients into three categories: normal, decreased and increased based on a normal range of HCs (**Table S8**). Based on this analysis, the most decrease in T cell subsets is related to Tregs (67%), while the most increase is related to $CD8^+T_{EMRA}$ (37%) in SIgAD patients. There were no significant differences between severe and mild phenotypes T-cell subsets (**Table S9**). Flow cytometry results of B cell and T cell subsets in 30 SIgAD patients have shown separately in Table **S10**and **S11** .

T cell proliferation

The data generated by CFSE labeled cultures was analyzed to quantify $CD4^+$ T cell proliferation. There was no significant difference in division index (DI), proliferation index (PI) and percent divided (PD) between SIgAD patients and HCs (**Table 3**). Interestingly, when we compared these indexes between SIgAD patients with severe and mild phenotype, we found that the median DI and PD in severe SIgAD patients in comparison with mild cases were significantly abrogated [0.1 (0.08-0.4) vs. 0.5 (0.3-0.8), p =0.019 and 12.2 (8.4-26.4) vs. 42.5 (26.8-52.6), p =0.009, respectively]. However, there was no significant difference in PI between severe and mild groups (**Table 3**). On the other hand, comparisons of DI, PI and PD between SIgAD patients with and without consanguinity were not significant (**Table S12**).

Discussion

SIgAD is the most prevalent PID with various clinical manifestations. These patients have a different spectrum of clinical manifestations. Accordingly, immunologic investigations in patients with a different spectrum of clinical manifestations are helpful. The most prevalent clinical manifestation in PIDs, especially in SIgAD, is recurrent respiratory infections (14-16). We found pneumonia as the most frequent complication in our registered symptomatic patients. Recurrent respiratory infections commonly manifested in the form of upper respiratory tract infection and may remain undiagnosed for several years, however, some SIgAD patients manifest more severe phenotypes such as bronchiectasis or obliterate bronchiolitis which force immunological investigation in these patients (17). Given that recurrent respiratory infections have been reported as the

most important cause of morbidity and death in children with PIDs, especially primary antibody deficiencies (18, 19), early diagnosis and management of respiratory disorders associated with SIgAD is very important (20, 21).

It has been indicated that abnormalities in B cell subsets are observed in some SIgAD patients (4, 5). Our results indicated a significant increase in naïve and transitional B cells and a strong decrease in marginal zone-like and switched memory B-cells. This abnormal B cell pattern suggests defects in the terminal stages of B-cells differentiation, similar to CVID patients (22). Given that CVID and SIgAD share almost similar genetic background and may accumulate as multiple cases within a family, this resemblance is predictable.

We detected a reduction in marginal zone-like and switched memory B-cells especially in severe SIgAD patients, as has been previously reported (23). SIgAD patients, especially a group of patients with severe clinical manifestations (recurrent and intensive infection, and autoimmunity), have lower switched memory B-cells (23-25). It has been suggested that the decrease in switched memory B-cell subpopulation is due to defects in the level of antibody class-switching recombination (CSR) process, caused by enzymatic deficiency, or abnormalities in the cytokine networks and their receptors (23). Some SIgAD patients with severe phenotype progress to CVID, which reflects this subgroup of SIgAD may share with CVID common immune pathogenesis, particularly in the development of CSR step. Accordingly, switched memory B-cells are considered a diagnostic biomarker in patients (23). However, the frequency of switched memory B-cells is normal among children in our study population, and the reduction was observed more in adult patients; suggesting that aging probably leads to the progression of SIgAD to CVID, especially in patients with severe clinical manifestations (data not shown). On the other hand, marginal zone B cells are a specialized population of B cells that produce IgM for the protection against infections, especially encapsulated bacteria (26). Although previous studies have shown that the number of marginal zone-like B cells in SIgAD patients was not different compared to normal controls (27), nevertheless, we obtained a significant reduction in marginal zone-like B cells in our cases, similar to a previous report in CVID patients (28). Reducing marginal B cell subsets in other patients with antibody production defects could be associated with an increased risk of infection such as pneumonia and a decrease in serum IgM levels, similar to CVID patients (29).

We found increased $CD21^{low}$ B cells compared to control, mainly in severe SIgAD patients. Previous studies have reported an increase in $CD21^{low}$ B cells in both SIgAD (4) and CVID patients (30), and other autoimmune diseases (31). An increase in the number of $CD21^{low}$ cells is directly related not only to autoimmunity but also to infection (30). On the other hand, chronic exposure to viral infection may lead to the conversion of antigen-reactive B cells to unresponsiveness $CD21^{low}$ B cells (32). To clarify the cause of expanded $CD21^{low}$ B cells; it is necessary to make further investigations for this B cell subpopulation. Given the high subpopulation of $CD21^{low}$B cells in CVID patients and the progression of some patients with SIgAD to CVID, the severe group of SIgAD patients with increased $CD21^{low}$ B cells will more likely develop to CVID. Therefore, they need a more regular follow-up to assess the course of the disease.

Transitional B cells are at an intermediate stage in the development between bone marrow immature cells and mature B cells in the spleen (33). In the present study, we observed significantly increased transitional B cells in our SIgAD patients especially in severe SIgAD patients, although the number of transitional B cells in children with SIgAD was normal (data not shown). In contrast to previous studies that showed decreased transitional B cells (9, 23, 34), adult patients indicated slightly increased transitional B cells. Moreover, Lemarquis et al., showed a decrease in the functional activity of transitional B cells based on IL-10 production and CpG stimulation (34). Given defect in the terminal stages of B-cells in SIgAD, it seems that an increase in transitional B cells and naïve B cells of our patients is due to a compensatory mechanism that augment early B cell development. Regarding different results between our study and others, it seems that this difference is due to different selection process, as all of our patients were symptomatic, while others studied heterogeneously asymptomatic and symptomatic SIgAD patients.

Regarding T cell subsets, we observed decreased total $CD4^+$ T cells, Th1, Th2, Treg cells and increased T_{EMRA} in both $CD4^+$ and $CD8^+$ cells. Consistent with our results, previous studies have shown an increase and reduction in $CD8^+$and $CD4^+$ T lymphocytes population, respectively (5). Also, we found that central

memory in both CD4+ and $CD8^+$ T cells and effector memory $CD8^+$ T lymphocytes were decreased in SIgAD patients compared to HCs. We observed a significant increase in the T_{EMRA} cell subset in both $CD4^+$ and $CD8^+$ lymphocytes population, especially in severe SIgAD patients. T_{EMRA} is a third T cell memory subset in peripheral inflammatory tissues that express CD45RA but lack expression of CCR7 or CD27. In humans, T_{EMRA} cells accumulation is affected by chronic infections, such as CMV (35, 36). An increase in these terminated T cell subsets might be due to chronic cellular response to infections in these patients, however further studies need to be performed regarding this phenomenon. Consistent with our results, Nechvatalova et al. demonstrated expanded $CD4^+$ and $CD8^+$ T_{EMRA} cells in SIgAD patients that were related to CMV infection (37). We did not examine CMV infection in SIgAD patients, but an increase in the number of T_{EMRA} cells subset in our patients could be related to chronic infections.

Regulatory T cells play an important role in the production of IgA antibodies by transforming growth factor-beta (TGF-β) secretion (38-40). We found a significantly decreased Tregs in our patients consistent with previous studies published (41), although one study reported increased Tregs in SIgAD patients (37). It has also been reported a correlation between reduced Treg cells and the severity of SIgAD disease, especially in individuals with autoimmunity, and IgA CSR deficiency in patients with severe clinical manifestations (24, 41). The low frequency of Treg cells and other T cell subsets including Th1 and Th2 in our patients may be due to low thymic emigrants caused by defective thymopoiesis and or increased apoptosis of these cells (42).

T cell functional assay by mitogenic or antigenic stimulation is an important feature in the diagnosis of various immune disorders and immunodeficiencies (43). Traditionally, there is one protocol for evaluating the function of T cells based on uptake of [3h] thymidine following PHA stimulation that is a dangerous method due to using radioactive components. CFSE proliferation assay is a practical choice for evaluated T cell responses to an antigen or mitogen in PID patients, especially SIgAD for targeting further potential T cell defects analyses in these patients (44). So far, there are few reports of T-cell response defects in SIgAD patients. As expected, our study does not reveal any significant difference in T cell response between patients and controls. However, when we categorized patients into two groups based on severe and mild phenotypes, severe patients indicated decreased T cell proliferation compared to mild patients. This result could be important finding for categorizing SIgAD patients for knowing prognosis of the patient. Moreover, this indicates that SIgAD patients with defective T cell proliferation should be followed further for precise medical management. We recommend further studies for evaluation of T cell function for SIgAD patients based on severe and mild phenotypes in other studies.

Conclusions

Our results indicated significant abnormalities in B cell patterns similar to CVID patients. Given that CVID and severe forms of SIgAD share almost similar clinical and immunological phenotypes and most likely genetic background, this notion is predictable. Based on phenotype analyses, we observed some more abnormalities in SIgAD patients with severe phenotypes such as a high subpopulation of $CD21^{low}$ B cells and T cell proliferation defect. Accordingly, severe patients manifest a higher number of respiratory infections compared to mild SIgAD, with numerous numbers of those suffering from sinusitis, otitis, pneumonia and bronchiectasis, suggesting further follow up and more precise management in these patients. The findings of the present study suggest that the investigation of B and T cell subsets could be helpful for a better understanding of the pathogenesis and prognosis of the disease.

Compliance with Ethical Standards

The study was approved by the Ethics Committee of Tehran University of Medical and written informed consent was obtained from all the individuals (IR.TUMS.VCR.REC.1396.2018).

Consent to participate:

Informed consent was obtained from all individual participants or parents included in the study.

Consent to publish:

Patients signed informed consent regarding publishing their data.

Authors Contributions:

AA and RY conceived and designed the study. SD and BM participated in sample collection of the patient. YB, TMS, FTZ, SD and AH performed the experiments. GA and NR analyzed and interpreted the data. YB, SN and FS wrote the manuscript and arranged the figures. HA and RY contributed to reviewing and editing the paper. . All authors approved the final version.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding :

This work was supported by the Tehran University of Medical Sciences [33167].

Availability of data and materials:

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

References

1. Aghamohammadi A, Mohammadi J, Parvaneh N, Rezaei N, Moin M, Espanol T, et al. Progression of selective IgA deficiency to common variable immunodeficiency. International archives of allergy and immunology. 2008;147(2):87-92.

2. Yazdani R, Fatholahi M, Ganjalikhani-Hakemi M, Abolhassani H, Azizi G, Hamid KM, et al. Role of apoptosis in common variable immunodeficiency and selective immunoglobulin A deficiency. Molecular immunology. 2016;71:1-9.

3. Bagheri Y, Sanaei R, Yazdani R, Shekarabi M, Falak R, Mohammadi J, et al. The Heterogeneous Pathogenesis of Selective Immunoglobulin A Deficiency. International archives of allergy and immunology. 2019;179(3):232-46.

4. Nechvatalova J, Pikulova Z, Stikarovska D, Pesak S, Vlkova M, Litzman J. B-lymphocyte subpopulations in patients with selective IgA deficiency. Journal of clinical immunology. 2012;32(3):441-8.

5. Litzman J, Vlková M, Pikulová Z, Štikarovská D, Lokaj J. T and B lymphocyte subpopulations and activation/differentiation markers in patients with selective IgA deficiency. Clinical & Experimental Immunology. 2007;147(2):249-54.

6. Lemarquis AL, Einarsdottir HK, Kristjansdottir RN, Jonsdottir I, Ludviksson BR. Transitional B cells and TLR9 responses are defective in selective IgA deficiency. Frontiers in immunology. 2018;9:909.

7. Celiksoy M, Yildiran A. A comparison of B cell subsets in primary immune deficiencies that progress with antibody deficiency and age-matched healthy children. Allergologia et immunopathologia. 2016;44(4):331-40.

8. Marasco E, Farroni C, Cascioli S, Marcellini V, Scarsella M, Giorda E, et al. B-cell activation with CD40L or CpG measures the function of B-cell subsets and identifies specific defects in immunodeficient patients. European journal of immunology. 2017;47(1):131-43.

9. Lemarquis AL, Theodors F, Einarsdottir HK, Ludviksson BR. Mapping of Signaling Pathways Linked to sIgAD Reveals Impaired IL-21 Driven STAT3 B-Cell Activation. Front Immunol. 2019;10:403.

10. Borte S, Pan-Hammarstrom Q, Liu C, Sack U, Borte M, Wagner U, et al. Interleukin-21 restores immunoglobulin production ex vivo in patients with common variable immunodeficiency and selective IgA deficiency. Blood. 2009;114(19):4089-98.

11. Abolhassani H, Kiaee F, Tavakol M, Chavoshzadeh Z, Mahdaviani SA, Momen T, et al. Fourth Update on the Iranian National Registry of Primary Immunodeficiencies: Integration of Molecular Diagnosis. Journal of clinical immunology. 2018;38(7):816-32.

12. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J Allergy Clin Immunol Pract. 2019;7(6):1763-70.

13. Shad TM, Yousefi B, Amirifar P, Delavari S, Rae W, Kokhaei P, et al. Variable Abnormalities in T and B Cell Subsets in Ataxia Telangiectasia. Journal of Clinical Immunology. 2020:1-13.

14. Reisi M, Azizi G, Kiaee F, Masiha F, Shirzadi R, Momen T, et al. Evaluation of pulmonary complications in patients with primary immunodeficiency disorders. European annals of allergy and clinical immunology. 2017;49(3):122.

15. Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. Annual review of immunology. 2011;29:273-93.

16. Bagheri Y, Babaha F, Falak R, Yazdani R, Azizi G, Sadri M, et al. IL-10 induces TGF-β secretion, TGF-β receptor II upregulation, and IgA secretion in B cells. European Cytokine Network. 2019;30(3):107-13.

17. Ozkan H, Atlihan F, Genel F, Targan S, Gunvar T. IgA and/or IgG subclass deficiency in children with recurrent respiratory infections and its relationship with chronic pulmonary damage. J Investig Allergol Clin Immunol. 2005;15(1):69-74.

18. Tavakol M, Jamee M, Azizi G, Sadri H, Bagheri Y, Zaki-Dizaji M, et al. Diagnostic Approach to the Patients with Suspected Primary Immunodeficiency. Endocrine, metabolic & immune disorders drug targets. 2020;20(2):157-71.

19. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012;119(7):1650-7.

20. Yazdani R, Abolhassani H, Asgardoon M, Shaghaghi M, Modaresi M, Azizi G, et al. Infectious and Noninfectious Pulmonary Complications in Patients With Primary Immunodeficiency Disorders. Journal of investigational allergology & clinical immunology. 2017;27(4):213-24.

21. Ahmadi M, Nouri M, Babaloo Z, Farzadi L, Ghasemzadeh A, Hamdi K, et al. Intravenous immunoglobulin (IVIG) treatment modulates peripheral blood Th17 and regulatory T cells in recurrent miscarriage patients: Non randomized, open-label clinical trial. Immunology letters. 2017;192:12-9.

22. Yazdani R, Seify R, Ganjalikhani-Hakemi M, Abolhassani H, Eskandari N, Golsaz-Shirazi F, et al. Comparison of various classifications for patients with common variable immunodeficiency (CVID) using measurement of B-cell subsets. Allergol Immunopathol (Madr). 2017;45(2):183-92.

23. Aghamohammadi A, Abolhassani H, Biglari M, Abolmaali S, Moazzami K, Tabatabaeiyan M, et al. Analysis of switched memory B cells in patients with IgA deficiency. Int Arch Allergy Immunol. 2011;156(4):462-8.

24. Abolhassani H1, Gharib B1, Shahinpour S1, Masoom SN1, Havaei A1,, Mirminachi B1 AN, Torabi-Sagvand B1, Khazaei HA3, Mohammadi J4,, Rezaei N1 AA. Autoimmunity in Patients With Selective

IgA Deficiency. J Investig Allergol Clin Immunol. 2015;25(2):112-9.

25. Arkwright PD, Abinun M, Cant AJ. Autoimmunity in human primary immunodeficiency diseases. Blood, The Journal of the American Society of Hematology. 2002;99(8):2694-702.

26. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nature Reviews Immunology. 2013;13(2):118-32.

27. Bukowska-Straková K, Kowalczyk D, Baran J, Siedlar M, Kobylarz K, Zembala M. The B-cell compartment in the peripheral blood of children with different types of primary humoral immunodeficiency. Pediatric research. 2009;66(1):28-34.

28. Karaman S BES, Gülez N, Genel F. The Significance of B-cell Subsets in Patients with Unclassified Hypogammaglobulinemia and Association with Intravenous Immunoglobulin Replacement Requirement. IranJImmunol. 2018;15(1):1-13.

29. Patuzzo G, Mazzi F, Vella A, Ortolani R, Barbieri A, Tinazzi E, et al. Immunophenotypic analysis of B lymphocytes in patients with common variable immunodeficiency: identification of CD23 as a useful marker in the definition of the disease. ISRN Immunology. 2013;2013.

30. Patuzzo G, Barbieri A, Tinazzi E, Veneri D, Argentino G, Moretta F, et al. Autoimmunity and infection in common variable immunodeficiency (CVID). Autoimmun Rev. 2016;15(9):877-82.

31. Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G, et al. Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. Proceedings of the National Academy of Sciences. 2009;106(32):13451-6.

32. Isnardi I, Ng Y-S, Menard L, Meyers G, Saadoun D, Srdanovic I, et al. Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. Blood, The Journal of the American Society of Hematology. 2010;115(24):5026-36.

33. Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG, Lipsky PE. Identification and characterization of circulating human transitional B cells. Blood. 2005;105(11):4390-8.

34. Lemarquis AL, Einarsdottir HK, Kristjansdottir RN, Jonsdottir I, Ludviksson BR. Transitional B Cells and TLR9 Responses Are Defective in Selective IgA Deficiency. Frontiers in Immunology. 2018;9.

35. Willinger T, Freeman T, Hasegawa H, McMichael AJ, Callan MF. Molecular signatures distinguish human central memory from effector memory CD8 T cell subsets. J Immunol. 2005;175(9):5895-903.

36. Martin MD, Badovinac VP. Defining Memory CD8 T Cell. Front Immunol. 2018;9:2692.

37. Nechvatalova J, Pavlik T, Litzman J, Vlkova M. Terminally differentiated memory T cells are increased in patients with common variable immunodeficiency and selective IgA deficiency. Cent Eur J Immunol. 2017;42(3):244-51.

38. Cazac BB, Roes J. TGF-β receptor controls B cell responsiveness and induction of IgA in vivo. Immunity. 2000;13(4):443-51.

39. Van Vlasselaer P, Punnonen J, De Vries J. Transforming growth factor-beta directs IgA switching in human B cells. The Journal of Immunology. 1992;148(7):2062-7.

40. Cerutti A, Rescigno M. The biology of intestinal immunoglobulin A responses. Immunity. 2008;28(6):740-50.

41. Soheili H, Abolhassani H, Arandi N, Khazaei HA, Shahinpour S, Hirbod-Mobarakeh A, et al. Evaluation of natural regulatory T cells in subjects with selective IgA deficiency: from senior idea to novel opportunities. Int Arch Allergy Immunol. 2013;160(2):208-14.

42. Yazdani R, Fatholahi M, Ganjalikhani-Hakemi M, Abolhassani H, Azizi G, Hamid KM, et al. Role of apoptosis in common variable immunodeficiency and selective immunoglobulin A deficiency. Mol Immunol. 2016;71:1-9.

43. McCusker C, Warrington R. Primary immunodeficiency. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology. 2011;7 Suppl 1(Suppl 1):S11.

44. Marits P, Wikström A-C, Popadic D, Winqvist O, Thunberg S. Evaluation of T and B lymphocyte function in clinical practice using a flow cytometry based proliferation assay. Clinical immunology. 2014;153(2):332-42.

45. Blanco E, Pérez-Andrés M, Arriba-Méndez S, Serrano C, Criado I, Del Pino-Molina L, et al. Defects in memory B-cell and plasma cell subsets expressing different immunoglobulin-subclasses in patients with CVID and immunoglobulin subclass deficiencies. 2019.

Table1. Comparison of demographic, clinical and immunological data of SIgAD patients with severe and mild phenotypes

Parameter	**Severe (n=12)**	**Mild (n=18)**	***p*-value**
Age, year (IQR)	14 (6.3-34.5)	10 (7.7-14.2)	0.27
Age of onset, year (IQR)	8 (3-26)	3 (1.1-5.5)	0.05
Age of diagnosis, year (IQR)	9 (8-26)	8 (3.5-9)	0.06
Diagnostic delay, year (IQR)	3 (1-5)	0.9 (2-4.7)	0.8
Sex (Male/Female)	9/3	14/4	1.0
Otitis, N (%)	3 (25)	0 (0)	0.08
Pneumonia, N (%)	6 (50)	5 (27.7)	0.5
Sinusitis, N (%)	6 (50)	3 (16.6)	0.1
Severe infections, N (%)	5 (41.6)	3 (16.6)	0.4
Bronchiectasis, N (%)	1 (8.3)	0 (0)	1.0
Autoimmune, N (%)	1 (8.3)	0 (0)	0.4
Lymphoproliferative, N (%)	0 (0)	1 (5.5)	1.0
Allergy, N (%)	7 (58.3)	4 (22.2)	0.11
Oral ulcer, N (%)	1 (8.3)	1 (5.5)	1.0
Recurrent diarrhea, N (%)	0 (0)	4 (18.2)	0.12
Chronic diarrhea, N (%)	0 (0)	3 (13.6)	0.26
Lymphocytes, cell/ul (IQR)	3650 (2300-5550)	4650 (4100-6725)	0.059
Lymphocytes % of leukocytes (IQR)	43 (39-58)	70 (55-78)	0.61
Neutrophil % of leukocytes (IQR)	52.7 (46-63.7)	42.5 (32-51.5)	0.04
CD3 % of lymphocytes (IQR)	53 (50.2- 63)	65 (57-70)	0.08
CD4 % of lymphocytes (IQR)	33.5 (31.5-40.7)	36 (34-40.5)	0.38
CD8 % of lymphocytes (IQR)	17.5 (16.2-20.2)	26 (22-31)	0.02
CD19 % of lymphocytes (IQR)	15.5 (8.2-32.5)	16 (13.5-23)	0.81
IgG, mg/dl (IQR)	1630 (1180-1931)	1216 (793-1534)	0.22
IgA, mg/dl (IQR)	2 (0-5.5)	0.3 (0-4.0)	0.77
IgM, mg/dl (IQR)	80 (118-175)	59.5 (49-92)	0. 03*
IgE, IU/ml (IQR)	32.5 (2.5-180)	49.5 (16.5-75)	0.84

IQR: Range with 25th percentile and 75th percentile; N: number.*P<0.05* were considered significant.

Table 2. Comparison of the percentage of B cell and T cell subsets between SIgAD patients and healthy controls

Parameter	**SIgAD patients (n=30) Percentage**	**Healthy Controls (n=30) Percentage**	***p*-value**
Lymphocyte (cell/ul)	4495 (3175-6325)	732.6 (536.3-1132)	**<0.001***
$CD19^{+}$ B cells % of lymphocytes	11.2 (9.4-13.07)	7.2 (6-8.6)	**<0.001***

Parameter	SIgAD patients (n=30) Percentage	Healthy Controls (n=30) Percentage	p-value
Naïve B-cells** ($CD19^+$, CD27-, IgM^+, IgD^+)	71 (63.7-80)	66.5 (56.2-71.1)	**0.036***
Marginal zone-like B-cells** ($CD19^+$, $CD27^+$, IgM^{++}, IgD^+)	2.3 (2-3.5)	3.4 (2.3-4.8)	**0.022***
Switched memory B-cells** ($CD19^+$, $CD27^+$, IgM^-, IgD^-)	3.5 (1.9-5.5)	6 (3.5-8.4)	**0.006***
IgM-only memory B-cells** ($CD19^+$, $CD27^+$, IgM^{++}, IgD^-)	2 (0.75-4.1)	3.5 (1.4-6.4)	0.219
$CD21^{low}$ B-cells** ($CD19^+$, $CD21^{-/low}$, $CD38^{-/low}$, IgM^{+++})	2.3 (1.5-4.5)	1.5 (1-2.2)	0.071
Transitional B-cells** ($CD19^+$, $CD21^+$, $CD38^{++}$, IgM^+)	8 (3.6-13.5)	4.8 (2.6-9.5)	**0.032***
Plasmablasts ** ($CD19^+$, $CD21^{-/low}$, $CD38^{++/+++}$, IgM^-)	0.7 (0.5-1.4)	0.9 (0.5-1.2)	0.377
$CD4^+$ T cells % of lymphocytes	36.5 (30.8-41.2)	40.1 (37.3-47.2)	**0.038***
Naïve T cells** ($CD4^+$, $CD45RA^+$, $CCR7^+$)	57.1 (39.5-71.3)	50 (36.5-56.2)	0.090
Central memory T cells** ($CD4^+$, CD45RA-, $CCR7^+$)	11 (7.3-13.1)	24.5 (16-29)	**<0.001***
Effector memory T cells** ($CD4^+$, CD45RA-, $CCR7^-$)	16.5 (8.6-29.5)	19 (14-22.2)	0.657
T_{EMRA} T cells** ($CD4^+$, $CD45RA^+$, $CCR7^-$)	9.5 (5.7-16.4)	2 (1.3-6.2)	**<0.001***
Th1 T cells** ($CD4^+$, IFN-γ^+)	7.7 (5.2-9.9)	12 (7.8-16)	**0.002***
Th2 T cells** ($CD4^+$, IL-4^+)	0.3 (0.2-0.4)	0.6 (0.4-1.3)	**<0.001***
Th17 T cells** ($CD4^+$, IL-$17A^+$)	1.1 (0.9-1-8)	1.05 (0.7-1.52)	0.095
Regulatory T cells ** ($CD4^+$, $CD25^+$, $FoxP3^+$, $CD127^{-/low}$)	0.3 (0.03-0.7)	1.4 (1.1-1.6)	**<0.001***
$CD8^+$ T cells % of lymphocytes	25.9 (19.2-30.9)	22.8 (19.8-26.9)	0.162
Naïve T cells*** ($CD8^+$, $CD45RA^+$, $CCR7^+$)	44.2 (26.4-55)	48.5 (40-57.3)	0.141

Parameter	SIgAD patients (n=30) Percentage	Healthy Controls (n=30) Percentage	*p*-value
Central memory T cells*** ($CD8^+$, CD45RA-, $CCR7^+$)	0.6 (0.3-0.8)	3 (2-6)	**<0.001***
Effector memory T cells*** ($CD8^+$, CD45RA-, CCR7-)	12.3 (7.6-22.1)	23.9 (19.5-27.2)	**<0.001***
T_{EMRA} T cells*** ($CD8^+$, $CD45RA^+$, CCR7-)	44.1 (28.4-55.7)	24.4 (20-31)	**<0.001***

Data are reported as median (25th -75th interquartile range). *$p<0.05$ were considered significant. ** % of Total B or Total helper T cells. *** % of Total cytotoxic T cells.

Table 3. Comparison of the percentage of B cell and T cell subsets between severe SIgAD and mild SIgAD patients compared to Healthy controls

Parameter	Healthy Controls (n=30)	Severe SIgAD (n=7)	p-value	Mild SIgAD (n=30)	p-value
Lymphocyte (cell/ul)	732.6 (536.3-1132)	3650 (2300-5550)	**0.000***	4650 (4100-6725)	**0.000***
$CD19^+$ B cells % of lymphocytes	7.2 (6-8.6)	10.8 (9.6-13.2)	**0.000***	11.6 (8.7-13)	**0.000***
Naïve B-cells** ($CD19^+$, CD27-, IgM^+, IgD^+)	66.5 (56.2-71.1)	71 (65.2-81.9)	0.066	70.6 (62.6-80)	0.105
Marginal zone-like B-cells** ($CD19^+$, $CD27^+$, IgM^{++}, IgD^+)	3.4 (2.3-4.8)	2.2 (2-3)	**0.040***	2.5 (1.8-4.3)	0.085
Switched memory B-cells** ($CD19^+$, $CD27^+$, IgM^-, IgD^-)	6 (3.5-8.4)	2.7 (1.7-4.7)	**0.003***	3.6 (1.9-7.6)	0.090
IgM-only memory B-cells** ($CD19^+$, $CD27^+$, IgM^{++}, IgD^-)	3.5 (1.4-6.4)	2.1 (0.7-4.3)	0.435	2 (0.9-3.9)	0.240

Parameter	Healthy Controls (n=30)	Severe SIgAD (n=7)	p-value	Mild SIgAD (n=30)	p-value
$CD21^{low}$ B-cells** ($CD19^+$, $CD21^{-/low}$, $CD38^{-/low}$, IgM^{+++})	1.5 (1-2.2)	2.7 (1.6-5.6)	**0.025***	2 (1.2-4)	0.179
Transitional B-cells** ($CD19^+$, $CD21^+$, $CD38^{++}$, IgM^+)	4.8 (2.6-9.5)	7.3 (2-12.9)	0.411	10.7 (3.9-13.8)	**0.047***
Plasmablasts ($CD19^+$, $CD21^{-/low}$, $CD38^{++/+++}$, IgM^-)	0.9 (0.5-1.2)	0.7 (0.3-1.1)	0.162	0.9 (0.5-1.7)	0.859
$CD4^+$ T cells % of lymphocytes	40.1 (37.3-47.2)	36.1 (28.3-41.5)	**0.030***	37.3 (31.3-41.3)	0.186
Naïve T cells** ($CD4^+$, $CD45RA^+$, $CCR7^+$)	50 (36.5-56.2)	59.6 (36.4-75.2)	0.133	57.1 (39.5-66.9)	0.198
Central memory T cells** ($CD4^+$, CD45RA-, $CCR7^+$)	24.5 (16-29)	10.8 (9.3-16.2)	**0.026***	11.2 (6.7-13.1)	**0.000***
Effector memory T cells** ($CD4^+$, CD45RA-, $CCR7^-$)	19 (14-22.2)	21.5 (8.3-24.8)	0.967	15.7 (10.3-13.3)	0.544
T_{EMRA} T cells** ($CD4^+$, $CD45RA^+$, $CCR7^-$)	2 (1.3-6.2)	9.3 (4.4-15.3)	**0.001***	10 (6.1-17)	**0.000***
Th1 T cells** ($CD4^+$, IFN-γ^+)	12 (7.8-16)	7.5 (4.4-13)	0.081	7.7 (5.7-9)	**0.002***
Th2 T cells** ($CD4^+$, IL-4^+)	0.6 (0.4-1.3)	0.3 (0.2-0.5)	**0.011***	0.3 (0.2-0.4)	**0.001***
Th17 T cells** ($CD4^+$, IL-$17A^+$)	1.05 (0.7-1.52)	1.2 (0.8-1.7)	0.336	1.1 (1-1.8)	0.094
Regulatory T cells ** ($CD4^+$, $CD25^+$, $FoxP3^+$, $CD127^{-/low}$)	1.4 (1.1-1.6)	0.6 (0.1-1)	**0.001***	0.2 (0-0.5)	**0.000***

Parameter	Healthy Controls (n=30)	Severe SIgAD (n=7)	p-value	Mild SIgAD (n=30)	p-value
$CD8^+$ T cells % of lymphocytes	22.8 (19.8-26.9)	22.5 (17.1-29.9)	0.911	27.3 (21.2-32)	0.054
Naïve T cells*** ($CD8^+$, $CD45RA^+$, $CCR7^+$)	48.5 (40-57.3)	30.4 (21.1-27.6)	0.029	45 (32.6-66.7)	0.655
Central memory T cells*** ($CD8^+$, CD45RA-, $CCR7^+$)	3 (2-6)	0.6 (0.4-0.8)	**0.002***	0.5 (0.3-0.8)	**0.000***
Effector memory T cells*** ($CD8^+$, CD45RA-, CCR7-)	23.9 (19.5-27.2)	12.3 (6.6-23.5)	**0.007***	12.1 (7.7-22.7)	**0.002***
T_{EMRA} T cells*** ($CD8^+$, $CD45RA^+$, CCR7-)	24.4 (20-31)	46.2 (36.1-56.9)	**0.000***	43.2 (24.7-47)	**0.003***

Data are reported as median (25th -75th interquartile range). *p <0.05 were considered significant. ** % of Total B cells or Total helper T cells. *** % of Total cytotoxic T cells.

Table 4. Comparison T lymphocytes proliferation indexes

Parameters	SIgAD patients (n=30)	Healthy Control (n=30)	p-value	Severe phenotype (n
Division Index (DI)	0.5 (0.2-0.8)	0.6 (0.4-0.7)	0.475	0.1 (0.08-0.4)
Proliferation Index (PI)	1.2 (1-1.8)	1.4 (1.2-1.6)	0.246	1.06 (1-1.6)
Percent Divided (PD)	35.3 (19.9-51.6)	39 (32.7-44)	1.000	12.2 (8.4-26.4)

Data are presented as median IQR (25th-75th). A p -value less than 0.05 are regarded as significant.

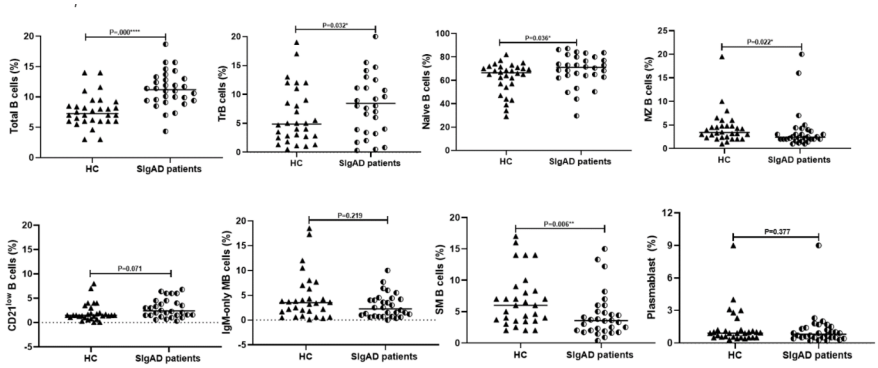

Figure1: Quantitative analysis of B cell and T cell subset percentages in SIgAD patients and Healthy controls . The median is represented by a horizontal line. Data were analyzed using the Mann–Whitney U test. $*p < 0.05$, statistical significance between patients and HCs. HC: Healthy control.

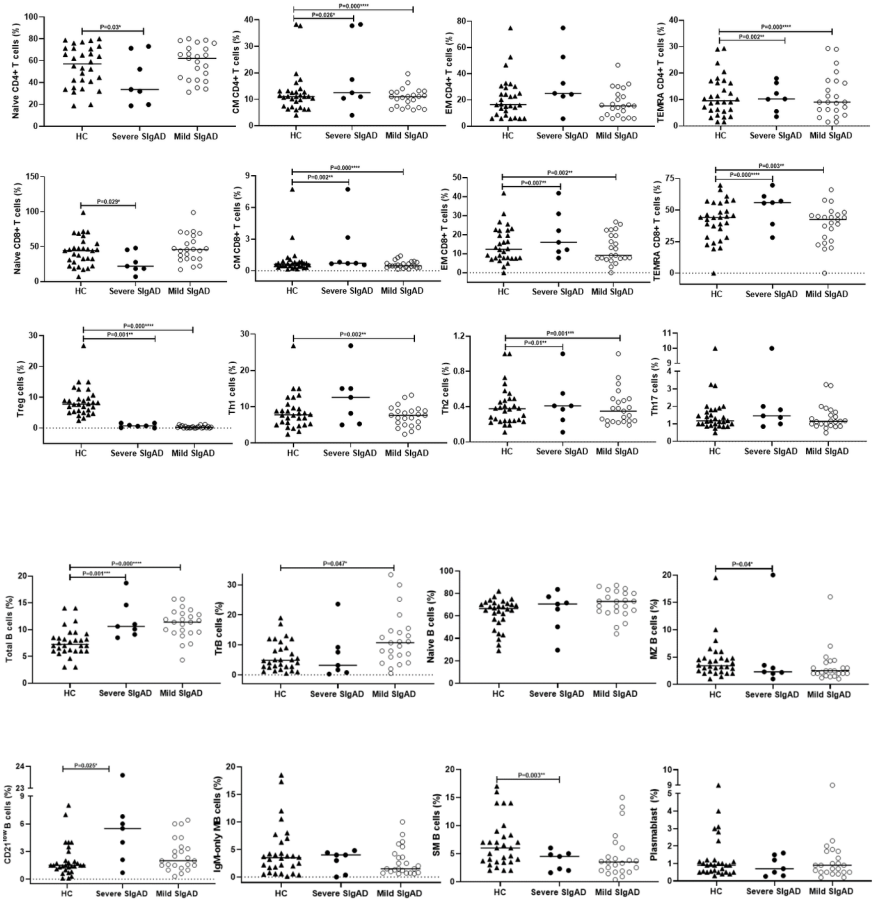

Figure 2: Quantitative analysis of B cell and T cell subset percentages in severe and mild SIgAD patients. The median is represented by a horizontal line. Data were analyzed using the Mann–Whitney U test. $*p < 0.05$, statistical significance between sever and mild patients

Supplementary data

B cells and T cells Abnormalities in Patients with Selective IgA Deficiency

Table S1. Panels of Antibodies Used for Staining of 100 ml Whole Blood.

Antigen	Fluorochrome	Clone	Company
Panel (24)	**Panel (24)**	**Panel (24)**	**Panel (24)**
CD19	APC	SJ25C1	eBioscience
IgM	PerCP-eFluor 710	SA-DA4	eBioscience
CD27	FITC	O323	eBioscience
IgD	PE	IA6-2	eBioscience
Panel (B2)	**Panel (B2)**	**Panel (B2)**	**Panel (B2)**

Antigen	**Fluorochrome**	**Clone**	**Company**
CD19	APC	SJ25C1	eBioscience
IgM	PerCP-eFluor 710	SA-DA4	eBioscience
CD38	FITC	HIT2	eBioscience
CD21	PE	HB5	eBioscience
Panel (T1)	**Panel (T1)**	**Panel (T1)**	**Panel (T1)**
CD4	PerCP-Cyanine5.5	RPA-T4	eBioscience
CD8a	PerCP-Cyanine5.5	RPA-T8	eBioscience
CD45RA	FITC	JS-83	eBioscience
CD197	PE	3D12	eBioscience
Panel (T2)	**Panel (T2)**	**Panel (T2)**	**Panel (T2)**
CD4	PerCP-Cyanine5.5	RPA-T4	eBioscience
CD25	APC	BC96	eBioscience
CD127	FITC	eBioRDR5	eBioscience
FOXP3	PE	236A/E7	eBioscience

Table S2. Demographic, clinical and immunologic features of SIgAD patients

Parameter	**Total SIgAD patient (n=30)**
Age, year (IQR)	11 (7.3-16)
Age of onset, year (IQR)	4 (2-8.75)
Age of diagnosis, year (IQR)	8 (5.87-13.75)
Diagnostic delay, year (IQR)	2 (1.1-4.75)
Sex (Male/Female)	23/7
Otitis, N(%)	3 (11.5)
Pneumonia, N(%)	11 (42.3)
Sinusitis, N(%)	9 (30)
Bronchiectasis, N(%)	1 (3.7)
Severe infections, N(%)	3 (10.3)
Autoimmune, N(%)	1 (3.3)
Lymphoproliferative, N(%)	1 (3.4)
Allergy, N(%)	11 (37.9)
Oral ulcer, N(%)	2 (6.9)
Recurrent diarrhea, N(%) (45)	4 (13.8)
Chronic diarrhea, N(%) (45)	3 (10.3)
Leukocyte, cell/ul (IQR)	4495 (3175-6325)
Lymphocytes, % of leukocytes (IQR)	69.5 (43-77.2)
Neutrophil, % of leukocytes (IQR)	49.25 (40.5-52)
CD3, % of lymphocytes (IQR)	62.5 (52-67.2)
CD4, % of lymphocytes (IQR)	36 (32.5-40.5)
CD8, % of lymphocytes (IQR)	21 (17.5-28)
CD19, % of lymphocytes (IQR)	16 (13.5-23)
IgG, mg/dl (IQR)	1303.5 (845.75-1812)
IgA, mg/dl (IQR)	0.3 (0-4)
IgM, mg/dl (IQR)	82 (51.25-130.75)
IgE, IU/ml (IQR)	32.5 (3.75-75)

Table S3. Demographic, clinical and immunologic features of SIgAD patients with and without consanguinity

Parameter	With Consanguinity (n=16)	Without Consanguinity (n=14)	*p*-va
Age (IQR)	9 (6.2-15.7)	12 (9.7-16.5)	0.338
Age of onset, year (IQR)	5.5 (2.2-21.3)	3 (0.4-8.7)	0.224
Age of diagnosis, year (IQR)	8 (7.2-21.7)	8.5 (5.1-13.7)	0.793
Diagnostic delay, year (IQR)	2 (0.5-3)	3.5 (1.3-8)	0.228
Sex (Male/Female)	13/3	10/4	-
Recurrent infections, N (45)(%)	3 (18.8)	5 (41.7)	0.401
Otitis, N (%)	2 (14.3)	1 (8.3)	1.000
Pneumonia, N (%)	4 (28.6)	7 (58.3)	0.126
Sinusitis, N (45)(%)	5 (31.3)	4 (28.6)	1.000
Bronchiectasis, N (%)	0 (0)	1 (8.3)	0.444
Respiratory infectious only, N (%)	2 (12.5)	2 (15.4)	1.000
Autoimmune, N (%)	1 (6.3)	0 (0)	1.000
Lymphoproliferative, N (%)(45)	0 (0)	1 (7.7)	0.448
Allergy, N (%)	4 (25)	7 (53.8)	0.143
Oral ulcer, N (%)	1 (6.3)	1 (7.7)	1.000
Recurrent diarrhea, N (%)	1 (6.3)	3 (23.1)	0.299
Chronic diarrhea, N (%)	0 (0)	3 (23.1)	0.078
Respiratory tract, N (%)	7 (43.8)	9 (64.3)	0.261
Gastrointestinal, N (%)	3 (18.8)	3 (21.4)	1.000
Neurologic, N (%)	0 (0)	2 (16.7)	0.188
Dermatologic, N (%)	3 (20)	3 (25)	1.000
Lymphocytes, % of leukocytes (IQR)	63 (41-77)	70 (50-78)	0.63
Neutrophil, % of leukocytes (IQR)	52.1 (38-63.5)	47 (43-51)	0.664
CD3, % of lymphocytes (IQR)	59 (51.2-69.7)	63.5 (53.7-67.2)	0.773
CD4, % of lymphocytes (IQR)	33.5 (32.2-40.7)	36 (33.5-40.5)	0.537
CD8, % of lymphocytes (IQR)	19.5 (16.5-24.7)	23 (19-31)	0.268
CD19, % of lymphocytes (IQR)	21 (15.2-35)	15 (9-18)	0.138
IgG, mg/dl (IQR)	1267 (824.5-1803.2)	1367 (1078.5-1978.2)	0.520
IgA, mg/dl (IQR)	0 (0-4)	3.5 (0-5.7)	0.299
IgM, mg/dl (IQR)	81 (50.7-120.5)	90 (50.7-164.5)	0.660
IgE, IU/ml (IQR)	29 (1.5-73)	60 (15-147.5)	0.250

IQR: Range with 25th percentile and 75th percentile; N: number. $*P<0.05$ were considered significant.

Table S4. Comparison of the percentage of B cell subsets between SIgAD patients with and without consanguinity

Β ëλλ Συβσετ (ςελλ/μλ)	**Percentage With Consanguinity (n=16)**	**Percentage Without Consanguinity (n=14)**	*p*-value
$CD19^+$ B cells % of lymphocytes	11.6 (10-14.3)	10.4 (8.7-12)	0.096
Naïve B cells ** ($CD19^+$, CD27-, IgM^+, IgD^+)	69.5 (62.2-79.2)	72.9 (64.1-82.3)	0.589

B ẽλλ Συβσετ (ςελλ/μλ)	Percentage	Percentage	p-value
Marginal zone-like B cells** ($CD19^+$, $CD27^+$, IgM^{++}, IgD^+)	2.4 (2-3)	2 (1.8-4.2)	0.912
Switched memory B cells** ($CD19^+$, $CD27^+$, IgM^-, IgD^-)	2.9 (1.9-4.9)	3.7 (1.8-7.1)	0.568
IgM-only memory B cells** ($CD19^+$, $CD27^+$, IgM^{++}, IgD^-)	3.8 (2.1-5.2)	1.2 (0.7-1.5)	**0.025***
$CD21^{low}$ B cells** ($CD19^+$, $CD21^{-/low}$, $CD38^{-/low}$, IgM^{+++})	2.7 (1.5-5.5)	2 (1.1-4.5)	0.496
Transitional B cells** ($CD19^+$, $CD21^+$, $CD38^{++}$, IgM^+)	7.8 (2.2-12.8)	9.6 (5.2-14.4)	0.430
Plasmablasts** ($CD19^+$, $CD21^{-/low}$, $CD38^{++/+++}$, IgM^-)	0.9 (0.5-1.7)	0.7 (0.4-0.9)	0.291

Data are reported as median (25th -75th interquartile range). *$p<0.05$ were considered significant. ** % of Total B cells.

Table S5. Distribution of normal increased and decreased B cell subsets in all SIgAD patients

B cell subsets	Normal N (%)	Increased N (%)	Decreased N (%)
$CD19^+$ B cells	26 (87%)	4 (13%)	0
Naïve B cells ($CD19^+$, CD27-, IgM^+, IgD^+)	21 (70%)	8 (27%)	1 (3%)
Marginal zone-like B cells ($CD19^+$, $CD27^+$, IgM^{++}, IgD^+)	26 (87%)	2 (7%)	2 (7%)
Switched memory B cells ($CD19^+$, $CD27^+$, IgM^-, IgD^-)	23 (77%)	0	7 (23%)
IgM-only memory B cells ($CD19^+$, $CD27^+$, IgM^{++}, IgD^-)	29 (97%)	0	1 (3%)
$CD21^{low}$ B cells ($CD19^+$, $CD21^{-/low}$, $CD38^{-/low}$, IgM^{+++})	29 (97%)	1 (3%)	0
Transitional B cells ($CD19^+$, $CD21^+$, $CD38^{++}$, IgM^+)	24 (80%)	4 (13%)	2 (7%)
Plasmablasts ($CD19^+$, $CD21^{-/low}$, $CD38^{++/+++}$, IgM^-)	26 (87%)	1 (3%)	3 (10%)

Table S6. Comparison of the percentage of B cell subsets between SIgAD patients with severe and mild and phenotypes

Parameter	**Severe SIgAD (n=7)**	**Mild SIgAD (n=30)**	***p*-value**
Lymphocytes, cell/ul (IQR)	3650 (2300-5550)	4650 (4100-6725)	0.059
CD19$^+$ B cells % of lymphocytes	10.6 (9.1-14.6)	11.4 (9.4-13)	0.922
Naïve B cells ** (CD19$^+$, CD27-, IgM$^+$, IgD$^+$)	70.5 (50.3-77)	72.9 (64-80)	0.540
Marginal zone-like B cells** (CD19$^+$, CD27$^+$, IgM^{++}, IgD$^+$)	2.2 (1.7-7.2)	2.5 (2-4)	0.957
Switched memory B cells** (CD19$^+$, CD27$^+$, IgM$^-$, IgD$^-$)	3.5 (1.9-5.2)	3.5 (1.9- 6)	0.957
IgM-only memory B cells** (CD19$^+$, CD27$^+$, IgM^{++}, IgD$^-$)	3.5 (0.2-4.1)	1.5 (0.8-4.2)	0.914
CD21^{low} B cells** (CD19$^+$, CD21-$^{/low}$, CD38-$^{/low}$, IgM^{+++})	5 (1.7-11)	2 (1.5-3.5)	**0.011***
Transitional B cells** (CD19$^+$, CD21$^+$, CD38^{++}, IgM$^+$)	2.4 (0.6-8)	10.7 (6-14.7)	0.106
Plasmablasts ** (CD19$^+$, CD21-$^{/low}$, CD38$^{++/+++}$, IgM$^-$)	0.6 (0.2-1.2)	0.9 (0.5-1.7)	0.387

Data are reported as median (25th -75th interquartile range). *p<0.05 were considered significant. ** % of Total B cells.

Table S7. Comparison of the percentage of T cell subsets between SIgAD patients with and without consanguinity

	T Cell Subsets	**Percentage With Consanguinity (n=16)**	**Percentage Without Consanguinity (n=14)**	***p*-value**
CD4$^+$ T cells	**CD4$^+$ T cells % of lymphocytes**	36.1 (30.5-41.5)	37.3 (30.4-41.9)	0.771
	Naïve T cells ** (CD4$^+$, CD45RA$^+$, CCR7$^+$)	64.8 (42.1-72.6)	50.8 (35-65.3)	0.339
	Central memory T cells** (CD4$^+$, CD45RA-, CCR7$^+$)	11.2 (9.9-13)	10.8 (6.2-13.4)	0.360

	T Cell Subsets	Percentage	Percentage	p-value
	Effector memory T cells** ($CD4^+$, CD45RA-, $CCR7^-$)	15.1 (5.8-24.8)	18.2 (14.8-30.7)	0.198
	T_{EMRA} T cells** ($CD4^+$, $CD45RA^+$, $CCR7^-$)	9.5 (5.5-16.2)	10 (5.6-18.6)	0.603
	Th1 T cells** ($CD4^+$, IFN-γ^+)	7.6 (6-9.5)	8.5 (4.2-11.1)	0.603
	Th2 T cells** ($CD4^+$, IL-4^+)	0.3 (0.2-0.4)	0.3 (0.2-0.6)	0.429
	Th17 T cells** ($CD4^+$, IL-$17A^+$)	1.1 (0.8-1.4)	1.4 (0.9-2.2)	0.134
	Regulatory T cells** ($CD4^+$, $CD25^+$, $FoxP3^+$, $CD127^{-/low}$)	0.5 (0.08-1.1)	0.2 (0-0.4)	0.050
$CD8^+$ T cells	**$CD8^+$ T cells % of lymphocytes**	23.8 (20.9-29.7)	29.6 (18-32)	0.329
	Naïve T cells *** ($CD8^+$, $CD45RA^+$, $CCR7^+$)	45.7 (29-47.9)	35.3 (21.9-66.5)	0.561
	Central memory T cells *** ($CD8^+$, CD45RA-, $CCR7^+$)	0.6 (0.4-1)	0.5 (0.3-0.7)	0.220
	Effector memory T cells *** ($CD8^+$, CD45RA-, CCR7-)	9 (7.5-15.8)	17.9 (7.6-22.7)	0.183
	T_{EMRA} T cells *** ($CD8^+$, $CD45RA^+$, CCR7-)	44.6 (30.7-55.9)	41.9 (25.1-51.1)	0.662

Data are reported as median (25th -75th interquartile range). $*p<0.05$ were considered significant. **% of Total helper T cells. *** % of Total cytotoxic T cells.

Table S8. Distribution of normal, increased and decreased proportions of T cell subsets in all SIgAD patients

	T Cell Subsets	Normal N (%)	Increased N (%)	Decreased N (%)
$CD4^+$ T cells	**$CD4^+$ T cells**	25 (83%)	3 (10%)	2 (7%)
	Naïve T cells ($CD4^+$, $CD45RA^+$, $CCR7^+$)	23 (77%)	6 (20%)	1 (3%)
	Central memory cells ($CD4^+$, CD45RA-, $CCR7^+$)	21 (70%)	1 (3%)	8 (27%)

	T Cell Subsets	Normal N (%)	Increased N (%)	Decreased N (%)
	Effector memory cells ($CD4^+$, CD45RA-, $CCR7^-$)	22 (73%)	0	8 (27%)
	T_{EMRA} cells ($CD4^+$, $CD45RA^+$, $CCR7^-$)	20 (67%)	10 (33%)	0
	Th1 ($CD4^+$, IFN-γ^+)	26 (87%)	1 (3%)	3 (10%)
	Th2 ($CD4^+$, IL-4^+)	27 (90%)	0	3 (10%)
	Th17 ($CD4^+$, IL-$17A^+$)	27 (90%)	3 (10%)	0
	Regulatory T cells ($CD4^+$, $CD25^+$, $FoxP3^+$, $CD127^{-/low}$)	10 (33%)	0	20 (67%)
$CD8^+$ T cells	**$CD8^+$ T cells**	28 (93%)	2 (7%)	0
	Naïve T cells ($CD8^+$, $CD45RA^+$, $CCR7^+$)	26 (87%)	1 (3%)	3 (10%)
	Central memory T cells ($CD8^+$, CD45RA-, $CCR7^+$)	27 (90%)	0	3 (10%)
	Effector memory T cells ($CD8^+$, CD45RA-, CCR7-)	25 (83%)	0	5 (17%)
	T_{EMRA} T cells ($CD8^+$, $CD45RA^+$, CCR7-)	18 (60%)	11 (37%)	1 (3%)

Table S9. Comparison of the percentage of T cell subsets between SIgAD patients with severe and mild phenotypes

	T Cell Subsets	Percentage	Percentage	p-value
		Severe SIgAD (n=7)	**Mild SIgAD (n=23)**	
$CD4^+$ T cells	**$CD4^+$ T cells % of lymphocytes**	38.4 (36.4-41.9)	35.8 (30.2-41)	0.477
	Naïve T cells ** ($CD4^+$, $CD45RA^+$, $CCR7^+$)	33.7 (19.9-71.4)	62.1 (42.3-71.3)	0.091
	Central memory T cells** ($CD4^+$, CD45RA-, $CCR7^+$)	12.5 (10.4-37.8)	11 (7.1-12.7)	0.220
	Effector memory T cells** ($CD4^+$, CD45RA-, $CCR7^-$)	25 (22.8-52.8)	15.4 (8.2-24.2)	**0.042***

	T Cell Subsets	Percentage	Percentage	*p*-value
	T_{EMRA} T cells** ($CD4^+$, $CD45RA^+$, $CCR7^-$)	10.2 (5.4-16.3)	9 (5.8-17)	0.787
	Th1 T cells** ($CD4^+$, IFN-γ^+)	12.6 (5.3-15)	7.6 (5-8.8)	0.082
	Th2 T cells** ($CD4^+$, IL-4^+)	0.4 (0.2-0.5)	0.3 (0.2-0.4)	0.589
	Th17 T cells** ($CD4^+$, IL-$17A^+$)	1.4 (1-2)	1.1 (0.9-1.6)	0.249
	Regulatory T cells** ($CD4^+$, $CD25^+$, $FoxP3^+$, $CD127^{-/low}$)	0.7 (0.1-1.6)	0.2 (0-0.5)	0.072
$CD8^+$ Tcells	**$CD8^+$ T cells % of lymphocytes**	21.5 (16.1-28.6)	27.8 (19.4-31)	0.122
	Naïve T cells *** ($CD8^+$, $CD45RA^+$, $CCR7^+$)	22.1 (18.6-45.6)	45.8 (32.7-65.7)	**0.020***
	Central memory T cells *** ($CD8^+$, CD45RA-, $CCR7^+$)	0.7 (0.6-3.1)	0.4 (0.3-0.8)	**0.024***
	Effector memory T cells *** ($CD8^+$, CD45RA-, CCR7-)	16 (11.1-31)	9.1 (7.5-19.6)	0.148
	T_{EMRA} T cells *** ($CD8^+$, $CD45RA^+$, CCR7-)	56 (38.9-61)	42.5 (25.5-46.3)	**0.044***

Data are reported as median (25th -75th interquartile range). *p<0.05 were considered significant. **% of Total helper T cells. *** % of Total cytotoxic T cells.

Table S10: Flowcytometry results of B cell subsets in 30 SIgAD Patients

Major Clinical Manifestations	**Plasmablasts** [c]	**CD21 low** [c]	**Tr B** [c]	**IgM only memory** [c]	**SMB** [c]	**MZB** [c]	**B Naïve** [c]	**BCD19** [b]	**Lymph** [a]	**Phenotype**	**Age**	**S**
Pneumonia URI	0.5	1.5	2	0.6	3.5	1.4	80	11.4	79	Mild	14	M
Pneumonia Rash Allergy	2.3	3.3	13	3.6	2.5	2	76.5	11.9	70	Mild	5	M

Major Clinical Manifestations	Plasmablasts [c]	CD21 low [c]	Tr B [c]	IgM only memory [c]	SMB [c]	MZB [c]	B Naïve [c]	BCD19 [b]	Lymph [a]	Phenotype	Age	S
Pneumonia Recurrent infection Allergy	1.8	2.5	30	7.7	2.5	2.2	68.5	13.7	89	Sever	6	M
Allergy	0.9	4.5	0.5	1	13.3	2	49.7	13	43	Mild	18	M
Recurrent infection Allergy Tonsillectomy	0.26	6.8	3.2	0	2.3	3	71.5	18.7	43	Sever	39	M
Allergy FTT	2	3	20	5.5	7	5	62	15.7	76	Mild	9	M
Sinusitis Aphthous stomatitis Recurrent cold	1.7	3.5	12.5	6.4	12.2	4.3	53.2	12.8	85	Mild	8	F
Otitis Sinusitis Autoimmunity Allergy	0.7	0.7	9.2	4	1.6	2.3	83.6	11	58	Sever	16	M
Pneumonia Recurrent infection Allergy	0.9	1.5	14.1	1.3	3	2.5	73	9.4	30	Sever	6	M

Major Clinical Manifestations	Plasmablasts [c]	CD21 low [c]	Tr B [c]	IgM only memory [c]	SMB [c]	MZB [c]	B Naïve [c]	BCD19 [b]	Lymph [a]	Phenotype	Age	S
Recurrent Sinusitis Pneumonia Aphthous stomatitis Allergy Recurrent infection	0.7	1.2	7	0.7	0.38	2	87.2	11	21	Sever	12	M
Thyroid abnormality Eye, nail and skin infection	1.5	6	1.7	3	4.83	1	70.5	9.11	31	Sever	46	F
Cold Cough	1.3	6	8	6	4.11	2.5	62.8	7.34	40	Mild	7	M
Asymptomatic	0.2	1.5	3.9	2	1.9	3	80	15.7	71	Mild	9	M
Pneumonia, Eyes infection, Otitis media, Diarrhea	0.3	23.6	0.8	0.36	6	20	29.5	10.6	41	Sever	38.7	M
Recurrent Sinusitis Gastrointestinal disorder	0.5	4	0.3	4	2	2	77	14.6	39	Sever	7.5	M

Major Clinical Manifestations	Plasmablasts [c]	CD21 low [c]	Tr B [c]	IgM only memory [c]	SMB [c]	MZB [c]	B Naïve [c]	BCD19 [b]	Lymph [a]	Phenotype	Age	S
Vitiligo *Asthma* Sinusitis	0.9	3	9.6	10	3.8	3	64.2	11.7	70	Sever	10	F
Diarrhea Allergy UTI	0.6	1	3.4	0.6	2	1.2	83.4	11.9	74	Mild	12	F
Epilepsy Asthma Pneumonia Allergy, Otitis media, Sinusitis	0.2	2	6.6	0.7	1.7	1.5	84.3	9.56	66	Sever	22	F
Diarrhea	0.5	0.3	15.5	2.5	0.91	1	86.3	9.4	69	Mild	10	M
Asthma Allergy							66	8.5	87	Mild	27	M
Cough	9	1.6	6	3.5	1.71	2.5	68.3	10	78	Mild	15	M
Allergy Diarrhea	1.7	2.3	33.4	2.5	3.7	2	72.9	12.4	67	Mild	4.8	F
Asthma Allergy Diarrhea	0.5	0.6	11.1	0.7	1.44	1.6	82	11.4	81	Mild	12	M
Diarrhea	1	6	10.7	1.5	15	16	44	8.84	76	Mild	10	M
Pneumonia Diarrhea	1	2	8	1.5	6	2	73.9	4.34	77	Mild	14	M
Asthma Allergy Pneumonia	0.4	4.5	25.2	1.2	4.4	4	68.8	9.9	70	Sever	16	M
Eyes infection Abscess	1.2	2.1	7.6	4.4	5	2.2	50.3	10.1	45	Sever	5	M
Allergy Pneumonia	0.4	6.4	4	0.8	8.2	4.4	73	14.3	86	Mild	9	M
Pneumonia Diarrhea	0.7	1.6	11.1	4.2	3.3	3	65	13.3	55	Mild	5	F
Allergy	0.5	1	14.7	1.5	3.6	7	64	7	53	Mild	16	M

Major Clinical Manifestations	**Plasmablast** [c]	**CD21 low** [c]	**Tr B** [c]	**IgM only memory** [c]	**SMB** [c]	**MZB** [c]	**B Naïve** [c]	**BCD19** [b]	**Lymph** [a]	**Phenotype**	**Age**	**S**
[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[a]:% of total peripheral blood mononuclear cells, [b]: % of Lymphocyte population, [c]: % of CD8+ T cells, [d]: % of CD4+ T cells, [e]: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower than normal range	[illegible]

Major Clinical Manifestations	Plasmablasts [c]	CD21 low [c]	Tr B [c]	IgM only memory [c]	SMB [c]	MZB [c]	B Naïve [c]	BCD19 [b]	Lymph [a]	Phenotype	Age	S

Table S11: Flowcytometry results of T cell subsets in 30 SIgAD Patients

Major Clinical Manifestations	Th17	Th2	Th1	Treg [d] (CD127$^{-/low}$)	T4 Effector memory [d]	T4 TEMRA [d]	T4 Central Memory [d]	T4 Naïve [d]	TCD4 [b]	T8 Effector memory [c]	T8 TEMRA [c]	T8 Central Memory [c]	T8 Naïve [c]	TCD8 [b]	Ly [a]
Pneumonia URI sore throat	1.18	0.49	6.8	1.2	5.8	1.5	12.7	80	51.6	7.82	19.2	1.25	71.8	24.2	79
Pneumonia Rash Allergy	1.15	0.49	7.65	0.3	5.8	17.2	11.4	65.7	31.7	9.16	57.9	0.29	32.7	30.1	70
Pneumonia Recurrent infection Allergy	0.84	0.47	6.93	1.1	5.6	8.6	9.8	76	35.9	4.87	48.2	0.47	46.4	23.5	89
Allergy	0.88	0.23	8.77	0	46.6	9	13.2	31.2	31	23.1	44	0.3	32.6	31.5	43
Recurrent infection Allergy Tonsillectomy	1.81	0.55	8.19	1.6	25	3.5	37.8	33.7	19.6	41.8	28.3	7.74	22.1	13.8	43
Allergy FTT	1	0.19	7.64	0	30.6	17	10.2	42.1	25.4	32	32	1.07	98.9	21.9	76

Major Clinical Manifestations	Th17	Th2	Th1	Treg[d] (CD127$^{-/low}$)	T4 Effector memory[d]	T4 TEMRA[d]	T4 Central Memory[d]	T4 Naïve[d]	TCD4[b]	T8 Effector memory[c]	T8 TEMRA[c]	T8 Central Memory[c]	T8 Naïve[c]	TCD8[b]	Ly[a]
Sinusitis	1.17	0.23	7.89	0	5.8	3.1	12.7	78.4	30.2	26.7	28.5	0.48	44.3	26.8	85
Aphthous stomatitis															
Recurrent cold															
Otitis Sinusitis	1	0.37	12.6	0.9	5.7	5.5	17.5	71.4	41.9	12.1	38.9	0.83	48.1	20.8	58
Autoimmunity Allergy															
Pneumonia	0.49	1	2.43	0.2	8.8	3.3	9.2	78.8	28.9	6.28	23.9	0.71	69.1	17.1	30
Recurrent infection															
Allergy															

Major Clinical Manifestations	Th17	Th2	Th1	Treg[d] (CD127$^{-/low}$)	T4 Effector memory[d]	T4 TEMRA[d]	T4 Central Memory[d]	T4 Naïve[d]	TCD4[b]	T8 Effector memory[c]	T8 TEMRA[c]	T8 Central Memory[c]	T8 Naïve[c]	TCD8[b]	Ly[a]
Recurrent Sinusitis	2	0.24	4.31	0.1	8.3	4.1	10.7	77	27.3	3.08	38.9	0.67	57.3	17.2	21
Pneumonia															
Aphthous stomatitis															
Allergy															
Recurrent infection															
thyroid abnormality	1.37	0.41	15	0.7	52.8	16.3	11	19.9	36.4	16	55.6	0.67	27.8	16.1	31
Eye, nail and skin infection															
Cold Cough	0.86	0.45	5.85	0.4	13.5	9	6.9	70.7	38.9	7.79	37.4	0.46	54.3	27.8	40
Asymptomatic	0.75	0.38	5.64	1.1	22.5	29	6.2	42.3	31.5	7.51	45	0.14	47.3	30.9	71
Pneumonia, Eyes infection, Otitis media, Diarrhea	2	0.11	26.8	0.63	32.8	10.1	38.3	18.8	48.8	22.1	56	3.16	18.7	30.6	41

Major Clinical Manifestations	Th17	Th2	Th1	Treg[d] (CD127$^{-/low}$)	T4 Effector memory[d]	T4 TEMRA[d]	T4 Central Memory[d]	T4 Naïve[d]	TCD4[b]	T8 Effector memory[c]	T8 TEMRA[c]	T8 Central Memory[c]	T8 Naïve[c]	TCD8[b]	Ly[a]
Recurrent Sinusitis	1.46	0.25	5	0	22.8	12.3	12.5	52.3	38.4	11.1	69.8	0.56	18.6	28.6	39
Gastrointestinal disorder															
Vitiligo *Asthma* Sinusitis	1.67	0.24	3.19	0	20.2	20.5	6.2	53.1	28.2	22.4	44.3	0.22	33	29	70
Diarrhea Allergy UTI	1.3	0.73	5	0.64	15	11.3	11.6	62.1	39.4	6.98	22.5	0.55	69.9	33.7	74
Epilepsy Asthma Pneumonia Allergy, Otitis media, Sinusitis	0.91	0.58	12.6	0.8	24.2	23.8	7.4	44.6	44.8	12.6	66.2	0.44	20.8	30.9	66
Diarrhea	1.13	0.19	9.65	0	16.8	6.2	13.1	63.9	27.4	7.51	46.3	0.41	45.8	36	69
Asthma Allergy	10	1	15	0.1	75	18	4	32	38	31	61	0.7	7	25	87
Cough	1	0.39	6.93	0.2	11.7	5.9	11.1	71.3	52.2	9.03	42.5	0.88	47.6	23.1	78
Allergy Diarrhea	1	0.38	8.88	0.2	15.4	13.5	6	65.1	53.6	16.4	45.7	0.3	37.6	18.3	67
Asthma Allergy Diarrhea	3.25	0.24	8.8	0.4	32.2	7.1	19.7	41	39.1	19.6	39.8	1.42	39.2	19.4	81
Diarrhea	3.19	0.3	4	0.5	30.3	29.3	6.3	34.1	36.6	25.5	57.1	0.17	17.2	43	76
Pneumonia Diarrhea	1.41	0.28	8.26	0	29.3	11.1	11.1	48.5	35.6	19.5	49.1	0.34	31.1	34.6	77

Major Clinical Manifestations	Th17	Th2	Th1	Treg[d] (CD127$^{-/low}$)	T4 Effector memory[d]	T4 TEMRA[d]	T4 Central Memory[d]	T4 Naïve[d]	TCD4[b]	T8 Effector memory[c]	T8 TEMRA[c]	T8 Central Memory[c]	T8 Naïve[c]	TCD8[b]	Ly[a]
Asthma Allergy Pneumonia	1.12	0.35	13.2	0.3	16.2	6.2	11	66.2	32.3	13.2	35.2	0.45	22.3	30.2	70
Eyes infection Abscess	0.85	0.41	5.3	1.6	24.3	10.2	10.4	73.1	40.4	7.7	57.2	0.72	45.6	21.5	45
Allergy Pneumonia	1.5	0.66	10.7	0	14.2	9	16.3	35.3	50.2	22.6	25.5	0.9	65.7	17.3	86
Pneumonia Diarrhea	1.8	0.22	9.4	0.5	6.3	16.2	7.1	55.2	35.8	15.2	44.2	0.66	44.2	18.6	55
Allergy	1.9	0.26	4.7	0.2	16	1.5	14	59	41	7.9	20	0.8	71	31	53

Major Clinical Manifestations	Th17	Th2	Th1	Treg [d] (CD127+/low)	T4 Effector memory [d]	T4 TEMRA [d]	T4 Central Memory [d]	T4 Naïve [d]	TCD4 [b]	T8 Effector memory [c]	T8 TEMRA [c]	T8 Central Memory [c]	T8 Naïve [c]	TCD8 [b]	Ly [a]
a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of total peripheral blood mononuclear cells, b: % of Lymphocyte population, c: % of CD8+ T cells, d: % of CD4+ T cells, e: % of CD19+ B cells White box: Normal, Red box: Higher than normal range, Blue box: Lower	a:% of to tal pe rip era blo mo cle cel b: % of Ly ph cy po u- la- tio c: % of Cl T cel d: % of Cl T cel e: % of Cl B cel W bo No ma Re bo Hi th no ma ran Bl bo Lo

Major Clinical Manifestations	Th17	Th2	Th1	Treg[d] (CD127$^{+/low}$)	T4 Effector memory[d]	T4 TEMRA[d]	T4 Central Memory[d]	T4 Naïve[d]	TCD4[b]	T8 Effector memory[c]	T8 TEMRA[c]	T8 Central Memory[c]	T8 Naïve[c]	TCD8[b]	Ly[a]

Table S12. Comparison T lymphocytes proliferation indexes in SIgAD patients with and without consanguinity

Subset	With Consanguinity (n=16)	Without Consanguinity (n=14)	p-value
Division Index (DI)	0.46 (0.16-0.69)	0.61 (0.31-0.98)	0.143
Proliferation Index (PI)	1.2 (1-1.8)	1.3 (1-1.9)	0.699
Percent Divided (PD)	28.8 (16-51.4)	46.4 (29.6-56.4)	0.080

Data are presented as median IQR (25th-75th). p -value less than 0.05 are regarded significant.