Alterations of peripheral blood B cell subsets in Chinese patients with adult idiopathic inflammatory myopathies

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Abstract

Objective

Abnormalities and hyperactivation of B cells have been described in idiopathic inflammatory myopathies (IIM). However, little is known about changes in the homeostasis of peripheral blood B cells in adult IIM patients. The aim of this study was to identify phenotypic alterations of B cell subsets and their relation to the overall clinical profile.

Methods

Blood samples were collected from 25 adult IIM patients and 15 healthy controls. Peripheral B cell subsets were classified into non-switched memory B cells (CD19+CD27+lgD+), switched memory B cells (CD19+CD27+lgD−), double-negative (DN) memory B cells (CD19+CD27−lgD−) and naïve B cells (CD19+CD27−lgD+) based on their surface phenotype as measured by flow cytometry. The clinical profile of IIM and its correlation with B cell subsets was further evaluated.

Results

Frequencies of CD19+ B cells and naïve B cells were increased in adult IIM patients compared with healthy controls ($p=0.005$ and $p<0.001$, respectively) and the frequency of memory B cells was decreased ($p<0.001$). Moreover, patients with a rash had lower non-switched memory B cells proportion ($p=0.032$). Patients with anti-MDA5+ antibodies had higher CD19+ B cells proportion than anti-ARS+ patients ($p=0.046$). Patients who were not receiving treatment had elevated levels of CD19+ B cells and naïve B cells along with reduced non-switched memory B cells compared with patients who were receiving treatment ($p=0.021$, $p=0.036$ and $p=0.032$, respectively).

Conclusion

Our findings demonstrate abnormalities in the homeostasis of the B cell subsets present in adult IIM patients, characterised by expanded CD19+ B cells and naïve B cells but reduced memory B cells. Phenotypic abnormalities of B cell subsets are associated with the presence of a rash, with anti-MDA5 positivity and with treatment.

Key words

idiopathic inflammatory myopathies, B cell subsets, memory B cells, non-switched memory B cells, switched memory B cells

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Introduction

Idiopathic inflammatory myopathies (IIM) are a group of systemic autoimmune disorders, which are characterised by impaired muscle function and are associated with specific autoantibodies. Recent research has suggested that activation of the innate and adaptive immune responses is involved in the pathogenesis of IIM. Evidence is growing that B cell dysregulation provokes autoimmune response involving production of autoantibodies and cytokines, presentation of antigens and modulation of T cells and dendritic cell functions (1). Many studies have reported that 80% of IIM patients test positive for autoantibodies (2), including myositis-specific (MSAs) and myositis-associated autoantibodies (MAAs). MSAs, in particular, are valuable in the diagnosis of IIM and are associated with a unique clinical phenotype. B cells can also be detected in peripheral blood and perivascular infiltrate in dermatomyositis (DM) patients. Serum levels of B cell activating factor (BAFF) were significantly higher in patients who tested positive for anti-Jo-1 antibodies as well as in those who diagnosed with DM (3). Furthermore, B cell depletion after anti-CD20 treatment has proven to be an effective therapy for IIM (4).

B cells arise from haematopoietic stem cells in the bone marrow and undergo multiple gene recombination and selection processes before becoming mature and able to produce functional antibody molecules. The B cell population comprises heterogeneous subpopulations with different phenotypes. Specific cluster of differentiation (CD) markers such as CD19 and CD27 can be used to identify universal B cells and memory B cells. Naïve B cells are characterised by a lack of CD27 expression and display IgD on the cell surface. Immediately following the transformation of naïve B cells into memory B cells, when IgD continues to be displayed, the cells are defined as non-switched memory B cells (CD19+CD27+lgD+). On conversion of the surface IgD into IgG, IgA or IgE, it is defined as switched memory B cells (CD19+CD27+lgD–), which can be rapidly activated to produce high-affinity, epitope-specific antibodies on re-encountering an antigen through T-dependent responses in the lymphoid follicle. CD19+CD27-lgD- B cells term as double-negative (DN) memory B cells, which have Ig genes with mutations and have progressed through a mechanism of class-switching similar to that of CD27+IgD- switched memory B cells (5).

Alterations in B cell subsets have been reported in patients with systemic lupus erythematosus (SLE) (6), Sjögren's syndrome (SS) (7), rheumatoid arthritis (RA) (8) and systemic sclerosis (SSc) (9). However, studies focusing on changes in peripheral blood B cell subsets homeostasis in adult IIM are limited, particularly in Chinese IIM patients. The pathogenesis of IIM is different from other systemic autoimmune diseases, and the individual roles of different B cell subsets have not yet been elucidated. During the current study, we performed a phenotypic analysis of B cell subsets, including naïve B cells, memory B cells, switched memory B cells, non-switched memory B cells and DN memory B cells. The source of the cells under analysis was peripheral blood taken from Chinese IIM patients. Our aim was to analyse the phenotypic abnormalities of B cell subsets in peripheral blood of adult IIM patients, especially in relation to clinical profile.

Materials and methods

Patients

Twenty-five patients were recruited from the Department of Rheumatology of the First Affiliated Hospital of Xi'an Jiaotong University, China, between April 2018 and May 2021. Patients who were greater than 18 years of age, fulfilled 1975 Bohan and Peter criteria were included. Patients with other autoimmune diseases or cancer or infectious diseases were excluded. Fifteen age and gender-matched healthy controls were collected. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (no: XJTU1AF2020LSK-194).

Data collection

Demographics, clinical features and laboratory data of the patients were

obtained through a systemic record review. Myositis disease activity was evaluated using physician global disease activity recorded on a 10cm visual analogue scale (VAS) (10) and myositis disease activity assessment visual analogue scales (MYOACT) (11) established by the International Myositis Assessment and Clinical Studies (IMACS) group. Inactive disease was defined as meeting the following three criteria: CK≤200U/L, physician VAS=0, and MYOACT scores=0. Otherwise, the disease condition was considered to be active (12).

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation (Tianjinghaoyang biological company, China) and resuspended in Staining Buffer at a concentration of $1x10^6$cells/100ul. APC-labelled anti-CD19, FITC-labelled anti-lgD- and PE-labelled anti-CD27 antibodies (all from BD Biosciences, San Diego, CA, USA) were added, followed by incubation at room temperature for 30 min. Stained cell images were visualised using a FACS Canto II flow cytometer (BD Biosciences) and analysed using CellQuest software.

Using anti-CD27 and anti-IgD labelling, non-switched memory B cells (CD19+CD27+lgD+), switched memory B cells (CD19+CD27+lgD–), DN memory B cells (CD19+CD27–lgD–) and naïve B cells (CD19+CD27–lgD+) were distinguished. The total memory B cell population was considered to comprise non-switched memory B cells plus switched memory B cells and DN memory B cells.

Statistical analysis

Data evaluation and statistical analysis were performed using SPSS version 17.0 software (SPSS, Chicago, IL, USA). Variables were expressed as the mean ± standard deviation (SD) or median (range). Numerical data groups were compared by a two-tailed Student's t-test. Spearman's rank correlation was used to examine the relationship between two continuous variables. *p*-values <0.05 were considered significant.

Table I. Demographics and clinical characteristics of the patients with IIM.

	IIM patients (n=25)	Healthy controls (n=15)
Demographics		
Age (years), mean±SD	46.4 ± 13.8	43.1 ± 11.3
Male/Female, n/n	7/18	4/11
Disease duration (months), median (range)	12 (1-132)	NA
Clinical characteristics		
Rash, n (%)	19 (76.0)	NA
Fever, n (%)	8 (32.0)	NA
Arthritis, n (%)	12 (48.0)	NA
Interstitial lung disease, n (%)	17 (68.0)	NA
Dysphagia, n (%)	1 (4.0)	NA
Clinically amyopathic, n (%)	15 (60.0)	NA
CK(U/L), median (range)	55 (24-22441)	NA
Active disease, n (%)	22 (88.0)	NA
MSAs	24 (96.0)	NA
Anti-Jo-1, n (%)	6 (25.0)	
Anti-EJ, n (%)	1 (4.2)	
Anti-PL-7, n (%)	1 (4.2)	
Anti-PL-12, n (%)	1 (4.2)	
Anti-MDA5, n (%)	6 (25.0)	
Anti-Mi-2, n (%)	2 (8.3)	
Anti-NXP-2, n (%)	1 (4.2)	
Anti-TIF1-γ, n (%)	1 (4.2)	
Anti-Ro-52, n (%)	2 (8.3)	
Negative, n (%)	3 (14.3)	
Treatment (at time of sample)		NA
Glucocorticoids, n (%)	11 (44.0)	
Cumulative dose (mg), median (range) [1]	2080 (30.0-26512.5)	
Daily dose (mg), median (range) [1]	21.8 (6.4-59.4)	
Treatment duration (months), median (range)	3.2 (0.03-119)	
Cyclophosphamide, n (%)	1 (4.0)	
Cyclosporine, n (%)	1 (4.0)	
Mycophenolate Mofetil, n (%)	1 (4.0)	
Methotrexate, n (%)	1 (4.0)	
Leflunomide, n (%)	1 (4.0)	
Tofacitinib, n (%)	1 (4.0)	

CK: creatine kinase; MSAs: myositis specific autoantibodies; Anti-Jo-1: anti-histidyl; Anti-EJ: anti-glycyl; Anti-PL-7: anti-threonyl; Anti-PL-12: anti-alanyl; Anti-MDA5: anti–melanoma differentiation associated gene 5; Anti-NXP-2: anti-nuclear matrix protein-2; Anti-TIF1-γ: anti-transcriptional intermediary factor 1-γ.
[1]Converted to equivalent dose of prednisone.

Results

Demographics and clinical characteristics of adult IIM patients

Demographics and clinical information for adult IIM patients (n=25) and healthy controls (n=15) were summarised in Table I. The IIM cohort included patients with DM (n=23) and polymyositis (PM) (n=2). Mean age was 46.4±13.8 years and median disease duration was 12 (1-132) months. Female patients accounted for 72% (18/25) of the cohort. Autoantibodies could be detected in 96% (24/25) of patients, of whom 37.5% (9/24) tested positive for anti-aminoacyl tRNA synthetase antibodies (anti-ARS including anti-Jo-1, anti-EJ, anti-PL-7 and anti-PL-12) and 25.0% (6/24) tested positive for anti-MDA5 antibodies. According to the assessments of myositis disease activity, 88.0% (22/25) of patients could be considered to have active disease. Among the disease cohort, 56% (14/25) had received no treatment and 44.0% (11/25) had been treated with glucocorticoids only (n=5; 20%) or with glucocorticoids plus DMARDs (n=5; 20%), including cyclophosphamide, mycophenolate mofetil, cyclosporine, leflunomide and methotrexate. One patient (4.0%) received tofacitinib plus glucocorticoids.

Frequency of B cell subsets in adult IIM patients

We found increased total CD19+ B cells (14.8±11.1 *vs*. 7.7±2.3%; t=-3.1; p=0.005), and increased naïve B cells (82.4±10.1 *vs*. 68.1±10.3%; t=4.3; p<0.001), along with decreased memory B cells (15.7±10.1 *vs*. 31.2±9.0%; t=-4.9; p<0.001), including non-switched memory B cells (4.6±3.8 *vs*. 8.9±3.2%; t=-3.6; p=0.001) and switched memory B cells (9.6±7.0 *vs*. 19.5±7.1%; t=-4.3; p<0.001) in the patient cohort compared with healthy controls. There were no significant differences in the frequency of DN memory B cells (2.8±1.6 *vs*. 3.3±1.8%; t=-0.863; p=0.394) (Fig. 1).

Correlation between B cell subsets and clinical form in adult IIM patients

To identify certain B cell subsets correlated with distinct clinical features, we assessed presentation of rash, arthritis, muscle weakness, interstitial lung disease (ILD), MSAs status and disease activity in our patient cohort and correlated the occurrence of these symptoms with B cell subsets. Our findings showed that patients exhibiting a rash had lower proportions of non-switched memory B cells than those with no rash (3.7±3.5 *vs*. 7.5±3.5%; p=0.032; Fig. 2A). The frequency of CD19+ B cells was significantly higher in the anti-MDA5 antibody positive compared to those in the anti-ARS antibody positive patients (23.7±15.3 *vs*. 7.3±5.1%; p=0.046; Fig. 2B). No significant associations were found between arthritis, muscle weakness, ILD, disease activity and B cell subsets.

We further analysed correlations between B cell subsets and laboratory data. The frequency of naïve B cells correlated positively with serum levels of IgM (r=0.419; p=0.037; Fig. 2C) , while the frequency of switched memory B cells correlated negatively with serum levels of IgM (r=-0.452; p=0.023; Fig. 2D). No relationship was established between B cell subsets and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum ferritin, albumin (ALB), creatine kinase (CK) or IgG.

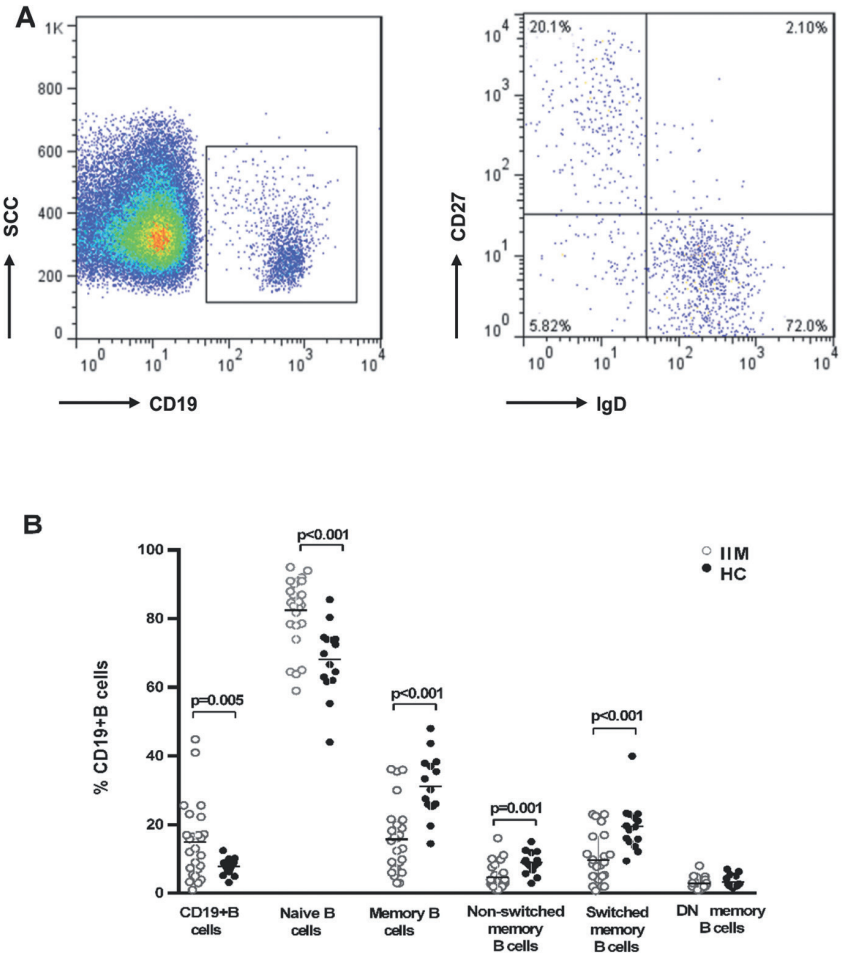

Fig. 1. Comparison of B cell subset proportions between adult IIM patients and healthy controls.
A. Using anti-CD27 and anti-IgD labelling, B cells were divided into naïve B cells (CD19+CD27-IgD+), non-switched memory B cells (CD19+CD27+IgD+), switched memory B cells (CD19+CD27+IgD-), double-negative (DN) memory B cells (CD19+CD27–IgD–), and memory B cells were defined as switched memory B cells plus non-switched memory B cell and double-negative memory B cells.
B. Scatter plots represent the percentages of total B cell and B cell subsets in 25 adult IIM patients (open circles) and 15 healthy controls (HC; closed circles). Horizontal lines indicate the mean of each set of values.

Effects of immunosuppressive therapy on distributions of B cell subsets in adult IIM patients

In order to assess whether the frequency of B cell subsets was influenced by the immunosuppressive therapy, we compared the composition of B cell subsets in patients receiving treatment and those with no treatment. 11 patients had received glucocorticoids only (n=5) or combined treatment (n=6) prior to our study. We showed a higher proportion of CD19+ B cells (19.3±12.3% *vs*. 9.2±5.9%; p=0.021) and naïve B cells (86.1±7.4% *vs*. 77.7±11.4%; p=0.036), and a lower proportion of non-switched memory B cells (3.1±2.1 *vs*. 6.7±4.6%; p=0.032) in untreated compared with treated patients (Fig. 3).

Discussion

In the present study, we found that alterations in the composition of the B cell subsets in adult IIM patients, characterised by expanded population of CD19+ B cells and naïve B cells along with reduced memory B cells. Moreover, phenotypic abnormalities of B cell subsets were associated with the presence of a rash, anti-MDA5 positivity and immunosuppressive treatment.

Our findings with regard to B cell distribution, are in agreement with previous studies (13-15). Using flow cytometric analysis of PBMCs, we found increased CD19+ B cells and naïve B cells plus decreased memory B cells. Loss of memory B cells is considered to stimulate dynamic expansion of naïve B cells

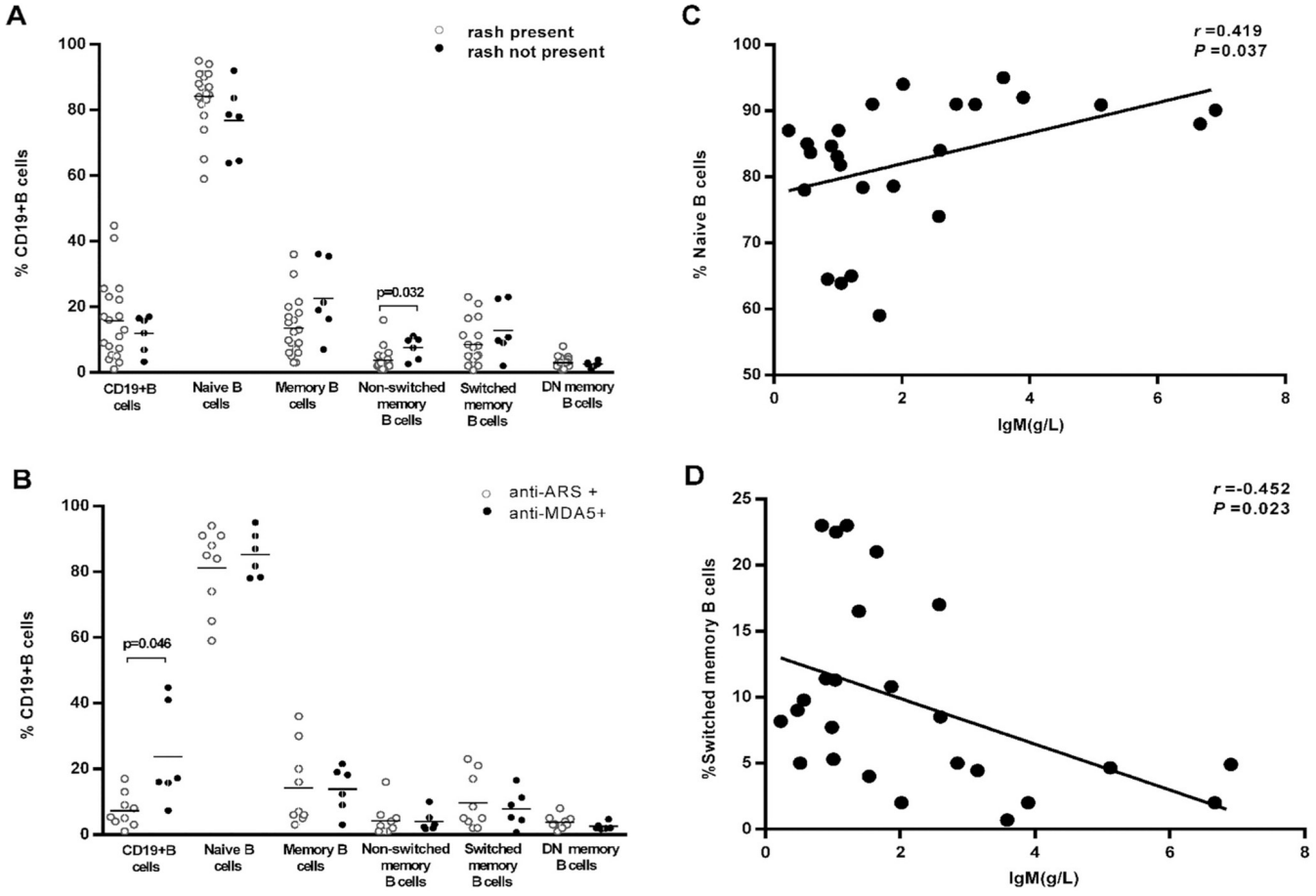

Fig. 2. Correlation of B cell subsets with different clinical profile in adult IIM patients.
A. Scatter plots represent the percentage values of B cell subsets between patients with rash (open circles, n=19) and without (closed circles, n=6).
B. Scatter plots represent the percentage values of B cell subsets between anti-ARS+ (open circles, n= 9) and anti-MDA5+ patients (closed circles, n=6). Horizontal lines indicate the mean of each set of values. **C.** Scatter plots show the correlations of the percentage of naïve B cells with serum levels of IgM.
D. Scatter plots show the correlations of the percentage of switched memory B cells with serum levels of IgM.

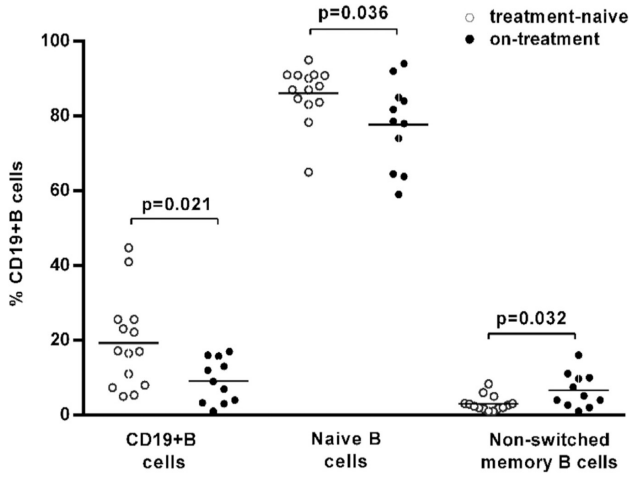

Fig. 3. Percentage of CD19+ B cells, naïve B cells and non-switched memory B cells in treatment-naïve and on-treatment patients. Scatter plots represent the percentage of CD19+ B cells, naïve B cells and non-switched memory B cells in 14 treatment-naïve (open circles) and 11 on-treatment (closed circles) patients.
Horizontal lines indicate the mean of each set of values.

in the bone marrow to maintain B cell homeostasis. We have previously obtained similar results in studies of SLE (16) and RA (17) patients. We present the novel finding that both non-switched memory B cells and switched memory B cells are reduced in the peripheral blood in IIM. Although the precise mechanism remains unclear, a possible explanation for decreased non-switched memory B cells is enhanced apoptosis due to proteolytic cleavage of CD27 from the cell surface (18), along with an increase in recruitment of auto-reactive B cells into lymphoid tissues (19). The reduction in the switched memory B cell population may be due to differentiation of these cells into IgG-secreting plasma cells or homing to target organs such as muscle. Therefore, these results may support the idea that naïve B cells, especially memory B cells, are a key component of the pathological features of IIM.

The current study is the first to correlate altered B cell subsets with clinical profile in adult IIM patients. We found decreased non-switched memory B cells in patients presenting with a rash compared with patients without. Non-switched memory B cells produce natural IgM, which contributes to the clearance of pathogenic immune complexes (20). It is possible that patients exhibiting a rash have impaired clearance of pathogenic immune complexes due to decrease in non-switched memory B cells, leading to persistent antigen stim-

ulation and immune inflammation. Further studies are necessary to elucidate the status of memory B cells in tissues, such as skin. We also showed elevated CD19+ B cells in anti-MDA5 antibodies positive patients compared with those who were positive for anti-ARS antibodies. Previous studies have demonstrated that the reduction of memory B cells was most pronounced in patients expressing anti-ARS (13,14). However, anti-MDA5 antibodies were not investigated in these studies. Isoda *et al*. reported that the prognosis of IIM patients for anti-MDA5 positive was much worse than those who were anti-ARS positive, especially in patients with ILD (21). Moreover, the frequency of CD19+ B cells have been reported to increase in active DM (22). Thus, it might be suggested that anti-MDA5 positive patients with increased CD19+ B cells are associated with poor outcomes, a conclusion which also described in patients with SLE (23).

Naïve B cells are transformed into activated B cells and induced to high levels of IgM production in the presence of CD4+ T cells and the stimulation of innate immune receptors such as Toll-like receptors (TLR) (24, 25). Switched memory B cells are believed to be post-germinal centre highly mutated memory B cells which undergo Ig isotype switching, resulting in secretion of IgG and IgA. The current study observed that serum levels of IgM were positively associated with the frequency of naïve B cells and negatively associated with the frequency of switched memory B cells. Further studies through a longitudinal cohort would be necessary to confirm it. Our investigations of the effects of concomitant or previous immunosuppressive therapy on B cell subsets have revealed higher frequencies of CD19+ B cells and naïve B cells and lower frequency of non-switched memory B cells in untreated compared with treated patients. Our findings support those of Piper *et al*. who reported an expansion of immature B cells and a reduction of memory B cells in pre-treatment patients of juvenile dermatomyositis (JDM) that was normalised by treatment (26). Sasaki *et al*. compared the B cell subsets of six anti-ARS positive patients with ILD before and after treatment and demonstrated an increase in memory B cells in all patients after treatment ($p<0.05$) while naïve B cells decreased in five patients ($p>0.05$) (15). In summary, these results support the idea that immunosuppressive therapy may influence the composition of the B cell population resulting in control of pathogenic autoantibodies production.

The present study has some limitations. First, a larger scale investigation, including a greater number of subjects would have more significance. We also recognise the need to expand our investigations to include more diverse B cell subsets and function analysis. Moreover, it is necessary to follow up the untreated patients in order to define the effect of treatment on immune signatures. Such studies would contribute greatly to clarify exact roles to different B cell subsets in adult IIM patients.

In conclusion, the present study confirms abnormalities in B cell subsets distribution in the peripheral blood of adult IIM patients, which were increased frequencies of CD19+ B cells and naïve B cells accompanied by decreased frequency of memory B cells. We have established that lower frequency of non-switched memory B cells is associated with the presence of a rash, and that higher frequency of CD19+ B cells is associated with anti-MDA5 positivity. These alterations and increased frequency of naïve B cells were found in untreated patients. Further studies are needed to identify mechanisms producing abnormal B cell subsets in adult IIM.

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