Peripheral blood lymphocytes and monocytes heterogeneity in SLE patients treated by Belimumab

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Abstract

Objective: To estimate the effect of Belimumab on the heterogeneity of peripheral blood lymphocytes and monocytes subsets in systemic lupus erythematosus (SLE).

Methods: There were three groups of populations, namely health controls (HC, n = 92), SLE patients treated (n = 26) and un-treated with BAFF (n = 155) in the present study. Cell subsets of $CD3^+T$ cells, $CD3^+CD4^+T$ cells, $CD3^+CD8^+T$ cells, $CD19^+B$ cells, $CD16^+CD56^+NK$ cells, $CD14^+HLA-DR^+$monocytes, $CD14^+CD206^+$monocytes and $CD14^+CD163^+$monocytes were estimated by flow cytometry.

Results: Compared with HC group, SLE patients had a higher level of $CD3^+T$ cells and $CD3^+CD8^+T$ cells, but a lower level of $CD3^+CD4^+T$ cells, $CD16^+CD56^+NK$ cells, $CD14^+CD206^+$monocytes, and $CD14^+CD163^+$monocytes. Besides, the ratio of $CD3^+CD4^+/CD3^+CD8^+T$ cells was much lower in SLE patients. Belimumab could effectively decrease $CD19^+B$ cells but increase $CD3^+T$ cell and $CD3^+CD8^+T$ cells in the peripheral blood of SLE patients. Moreover, SLE patients had decreased $CD14^+CD206^+$ monocytes and $CD14^+CD163^+$ monocytes in peripheral blood, while Belimumab therapy did not affect the heterogeneity of monocytes in SLE.

Conclusions: Our results revealed the heterogeneity of lymphocytes and monocytes in SLE patients. The clinical efficacy of Belimumab in SLE treatment is remarkable due to its significant effect on down-regulating B cell but up-regulating $CD3^+T$ cell and $CD3^+CD8^+T$ cells in SLE.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by excessive activation of lymphocytes, autoantibodies generation and immune complexes deposition, which thus causes multiple systems and organs injuries [1]. The clinical manifestations are heterogeneous among SLE patients [2], including cutaneous lupus, joint pain, lupus nephritis and neuropsychiatric manifestations, and so forth [3]. Women at childbearing age are more susceptible to SLE [3]. Genetic susceptibility and environmental factors have also been elucidated in the etiology of SLE [3, 4]. To the best of our knowledge, both innate and adaptive immune cells participate in SLE development and progression. Previous studies have demonstrated that the excessive clearance of apoptotic debris triggered abnormal activation of T cells and B cells mediated by type I interferons (IFN) and Tolllike receptors (TLRs), subsequently lead to a further cascaded expansion of immune and inflammatory responses [5, 6]. Abnormal activation of B cells and excessive antibodies production play critical roles in SLE [7]. Activated B cells can effectively present specific antigens to T cells and promote the activation of T cells. Abnormalities of $CD4^+$, $CD8^+$ and $CD4^-CD8^-$ T lymphocytes are also involved in regulating inflammation and immunity in SLE [8]. Abnormal activation of immune cells leads to the imbalance of immune homeostasis and the occurrence of autoimmune diseases ultimately [9, 10], suggesting the vital role of immune dysregulation in the pathogenesis of autoimmune diseases.

Excessive activation of the immune system and immune escape can result in autoimmune disorders and SLE occurrence. Imbalance of lymphocytes and mononuclear macrophages has been well documented in the pathogenesis of SLE [6, 11, 12]. Currently, Belimumab (BLyS-specific inhibitor) is a new try for SLE treatment, which is a humanized monoclonal antibody targeting B-cell activating factor (BAFF), namely B-lymphocyte stimulator (BLyS). BAFF is an essential factor for the survival, differentiation, activation of B cells [13]. Overexpression of BAFF can promote autoreactive B cells growth and activation [14]. Another study has verified the significant effect of Belimumab on B cells in SLE [15]. BAFF in the circulation of SLE patients is suggested to be closely associated with the disease activity [16, 17]. Therefore, anti-BAFF therapy may be promising for the treatment of SLE patients, particularly those

difficult to obtain clinical relief. In this study, we analyze the efficacy of Belimumab for SLE by estimating the heterogeneity and phenotypic transformation of immune cells, including $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $CD19^+$B cells, $CD16^+CD56^+$NK cells, $CD14^+HLA-DR^+$ monocytes, $CD14^+CD206^+$ monocytes and $CD14^+CD163^+$ monocytes. Findings in the present study will provide novel insight into understanding the therapeutic significance of anti-BAFF treatment among SLE patients.

1. Materials And Methods

1.1 Study population

A total of 140 SLE patients untreated with Belimumab, 26 SLE patients treated with Belimumab and 92 age- and gender-matched health controls (HC) were included in this study. All participants were recruited from the First Affiliated Hospital of Weifang Medical University between November 2019 and April 2020. This work was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Weifang Medical University. All subjects had read and signed a written informed consent prior to tests. SLE patients were diagnosed according to the 1997 classification criteria for SLE revised by American College of Rheumatology [18]. All cases had received no therapeutic intervention before study. Patients with malignant tumors or any other immune diseases are all excluded. Whether treatment with Belimumab or not during the study was regarded as a criterion for distinguishing treatment from non-treatment. Characteristics of all participants were summarized in Table 1. Clinical data collected only from SLE patients are as follows: erythrocyte sedimentation rate (ESR), serum concentrations of complement factors 3 (C3) and 4 (C4)、erythrocyte count (RBC), white blood cell count (WBC), platelet count (PLT)、micro-albminuria (mALB)、antinuclear antibody (ANA) and anti-dsDNA antibody.

1.2 Sample collection and preparation

Peripheral blood samples were collected from HC and SLE patients. Whole blood samples were incubated directly with fluorescence labeled antibodies. Specific antibodies of CD3-FITC, CD8-PE, CD45-PerCP, CD4-APC, CD16+CD56-PE, CD19-APC were purchased from Agilent Technologies Inc. (USA). Antibodies of CD14-FITC, HLA-DR-PE, CD206-PE, and CD163-APC (BioLegend, USA) were used for detecting mononuclear macrophages according to the concentration of reagent protocols. NoveCyte flow cytometry (Agilent, USA) was used to assay all samples. Final results of percentage and absolute count of positive markers were presented. The percentage and absolute count of $CD3^+$T cells, $CD3^+CD4^+$T cells, $CD3^+CD8^+$T cells, $CD19^+$B cells, $CD16^+CD56^+$ NK cells, ratio of $CD3^+CD4^+/CD3^+CD8^+$T cells, as well as percentage and mean fluorescence intensity (MFI) of $CD14^+HLA-DR^+$ monocytes、$CD14^+CD206^+$ monocytes and $CD14^+CD163^+$ monocytes were all estimated.

1.3 Statistical analysis

All statistical analyses were finished by GraphPad Prism (GraphPad Software, USA) and expressed as means±standard deviation (SD). Unpaired Student's t-test or paired Student's t-test was used for analysis. It is considered statistically significant, when *P-values* of two-tail were less than 0.05.

2. Results

2.1 Clinical parameters analysis in SLE patients with/without Belimumab therapy

Data between groups were analyzed, including non-paired comparison of untreated group (n=140) *vs* treatment group (n=26), paired comparison of pre-treatment group (n=17) *vs* post-treatment group (n=17) (Table 1). The mean age in

HC group was significantly different from untreated patients ($P < 0.05$), but no significant difference was discovered between HC group and the treated group ($P = 0.83$), and even between the treated group and untreated group ($P = 0.07$). In addition, we found statistical significance between the three groups in this study regarding the gender status (all P values less than 0.05).

Data with regard to ESR, C3, C4, RBC, WBC, PLT, mALB, ANA and anti-dsDNA antibody were summarized in Table 2. No significant difference was observed between the treated group and untreated group regarding all the clinical parameters except for peripheral blood PLT. The level of PLT in the treated group was higher than those in the untreated group (215±75.76 vs 247.5±54.8, $P<0.05$). However, statistically differences of several parameters could be found between the pre-treatment and post-treatment groups, including C3, C4, PLT and mALB (Table 2). Compared with SLE patients before treatment, the serum C3 and C4 levels in patients with SLE after Belimumab treatment (for C3, 0.61±0.26 vs 0.77±0.21, $P<0.05$; for C4, 0.13±0.09 vs 0.18±0.09, $P<0.05$) were much higher. In addition, the mean level of mALB in the post-treatment group was significantly lower than that the pre-treatment group (1410±1611 vs 686.0±1149, $P<0.05$). After treatment with Belimumab, the level of peripheral blood PLT was also increased than before treatment (213.7±63.45 vs 240.3±67.05, $P<0.05$). However, no difference was found in terms of ESR ($P=0.09$), WBC ($P=0.15$), RBC ($P=0.30$), ANA ($P=0.35$) and anti-dsDNA antibody ($P=0.29$) between the three groups.

2.2 Heterogeneity of lymphocyte subsets in SLE patients with/without Belimumab therapy

The frequencies of lymphocytes $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, $CD3^+CD4^+/CD3^+CD8^+$ T cells, $CD19^+$ B cells and $CD16^+CD56^+$ NK cells were evaluated by flow cytometry. All data of percentage and absolute count were summarized in Table 3 and Table 4. Figure 1 and Figure 2 also showed the differences between groups. Compared with the HC group, the frequency of $CD3^+$ T cells in SLE group was significantly higher, no matter in Belimumab-treated group or Belimumab-untreated group (Figure 1A, 3A and 3B). By contrast, the absolute count of $CD3^+$ T cells in the untreated group was lower than that in the HC group (Figure 2). Furthermore, both the percentage and absolute count of $CD3^+CD4^+$ T cells of SLE patients without Belimumab treatment were evidently lower than those in HC group, while the percentage of $CD3^+CD8^+$ T cells among the untreated patients was obviously higher than that in the HC group ($P<0.05$) (Figure 3A, 3B). In addition, an increased percentage of $CD3^+CD8^+$ T cells was found in SLE patients with Belimumab treatment ($P<0.05$), while no significant change regarding the percentage of $CD3^+CD4^+$ T cells was observed in SLE patients received Belimumab treatment when comparing with HC group (Figure 1A, 2, 3A and 3B). Besides, the ratio of $CD3^+CD4^+/CD3^+CD8^+$ T cells among SLE patients with or without Belimumab treatment was significantly decreased when comparing with the HC group (Table 3). Moreover, SLE patients undergoing the treatment with Belimumab had a distinct decline of $CD19^+$ B cells, regarding both percentage and absolute count of B cells when comparing with the HC group (Figure 1A, 2, and Figure 3C). Both the percentage and absolute count of $CD19^+$ B cells were significantly decreased in the Belimumab treated group compared with the Belimumab untreated group (Figure 1A, 2, 3C and Table 3). In addition, SLE patients treated and untreated with Belimumab had less $CD16^+CD56^+$ NK cells than healthy controls, while no significant difference was observed between SLE-treated group and SLE-untreated group (Figure 1A, 2, and Figure 3D). Taken together, lymphocytes are heterogeneous in SLE, and Belimumab therapy exerts modifying effects on the differentiation of peripheral blood lymphocytes.

2.3 Heterogeneity of monocytes in SLE patients with/without Belimumab therapy

Monocyte subsets were estimated in peripheral blood samples from 14 HC, 16 untreated- and 16 treated- SLE patients, including $CD14^+HLA-DR^+$ monocytes, $CD14^+CD206^+$ monocytes and $CD14^+CD163^+$ monocytes (Table 4). The percentages of $CD14^+CD206^+$ monocytes and $CD14^+CD163^+$ monocytes were significantly decreased in SLE patients

compared with healthy controls, respectively (Table 4 and Figure 4). However, there was no significant difference for CD14$^+$HLA-DR$^+$ monocytes between the three groups (Table 4). Our data only suggest a significant downregulation of M2 monocytes. In addition, the percentage and MFI of monocyte subsets between Belimumab treated group and Belimumab untreated group were not different (Table 4 and Figure 4). Taken together, SLE patients had decreased CD14$^+$CD206$^+$ monocytes and CD14$^+$CD163$^+$ monocytes in peripheral blood, namely M2 monocytes. Additionally, Belimumab therapy did not affect the heterogeneity of monocytes in SLE.

2.4 Heterogeneity of lymphocytes in SLE patients before and after the treatment of Belimumab

A total of 17 SLE patients receiving Belimumab treatment were selected for self-paired analysis before and after treatment of Belimumab. We found that the Belimumab treatment exerted inhibitory effects on CD19$^+$ B cell, characterized by significantly decreased percentage and absolute value of CD19$^+$ B cells (Table 5, Figure 5). Besides, the percentage of CD3$^+$ T cells was also obviously increased in peripheral blood samples from SLE patients after Belimumab treatment (Table 5). Moreover, no significant difference was found between the two groups regarding CD3$^+$CD4$^+$ T cells, CD3$^+$CD8$^+$ T cells, CD3$^+$CD4$^+$/CD3$^+$CD8$^+$ T cells and CD16$^+$CD56$^+$ NK cells (Table 5, Figure 5). We failed to estimate the effect of Belimumab on monocytes heterogeneity in SLE due to small sample size. All these results suggested that anti-BAFF treatment leaded to decreased CD19$^+$ B cells while increased CD3$^+$ T cells in SLE patients.

3. Discussion

Integral immune system and normal immune cell differentiation and polarization are essential for homeostasis. Abnormal lymphocytes and monocytes populations play important roles in the pathogenesis and disease progression of SLE. As a common autoimmune disease, SLE is characterized by the imbalance of lymphocyte and monocytes subsets as well as dysregulated cell functions [19-21]. BLyS is an activating factor for B lymphocytes, namely BAFF, which is crucial for the survival, differentiation, activation of B cells. It has been well documented that Belimumab can negatively regulate the activation of B cell by binding with soluble BLyS with high affinity and inhibiting its activity, whereas the efficacy of Belimumab still needs further investigation [22-24]. In the present study, we have found that Belimumab is an effective therapy for SLE patients, which can regulate the differentiation of B cells and lymphocytes in SLE.

B cells play a critical role in SLE pathogenesis [25]. Previous studies have suggested B cell dysfunction is closely related to SLE via epigenetic programming [26]. B cell deficiency results in excessive autoantibodies production, accumulation of immune complex and the activation of complement system. Wang et al. [27] have reported that the CD11c^{hi} T-bet$^+$ B cells are significantly increased in SLE, which can differentiate into autoreactive plasma cells and participate in SLE. Hyperactivation of CD19$^+$CD24^{hi}CD38^{hi} transitional B Cells are reported to be driven by upregulated TLR7, which elaborates their pathologic role in SLE [28]. Thus, inhibiting the differentiation and activation of B cells and plasma cells production is essential for SLE treatment. We have estimated the frequency of lymphocytes and monocytes subtypes in the peripheral blood of SLE patients with or without Belimumab treatment. The level of peripheral blood B cells in SLE patients receiving Belimumab treatment is much lower than that in those untreated patients. Besides, SLE patients in post-treated group have shown a significant decrease of B cells than the pretreated group. Nevertheless, the significant differences in B cell level between untreated SLE patients and HC were not found in our study.

Dysregulation of autoreactive B cells and T cells plays a critical role in SLE [5, 29]. A previous study has shown that high expression of OX40L in B cells results in augment of Tfh cells, which thus contributes to SLE [30]. Tfh cells are

responsible for regulating B cells [31]. B cells can mobilize T cells and thus initiate a series of destructive inflammatory events in SLE. Changes in immune cell frequencies and activities are closely related to the pathogenesis of SLE [32]. Similarly, imbalance of T cell subsets has been demonstrated among SLE patients in our work, characterized by decreased $CD3^+CD4^+$ T cells but increased $CD3^+CD8^+$ T cells in peripheral blood samples from SLE patients. Certain treatments can induce the heterogeneity of immune cells in SLE. A number of studies have documented that Belimumab exerts significant effects on B cells but not T cells [33, 34]. In our study, Belimumab significantly decreases the frequency of $CD19^+$ B cells in peripheral blood of SLE patients. Besides, Belimumab can promote $CD3^+$ T cells among treated SLE patients compared with Belimumab untreated patients. Moreover, no significant difference is observed regarding other T cell subsets as well as the ratio of $CD3^+CD4^+/CD3^+CD8^+$ T cells between different groups. Accordingly, the significant role of Belimumab in SLE treatment is probably attributed to its altering effect on $CD19^+$ B and $CD3^+$ T subtypes. Nevertheless, the underlying molecular mechanism needs to be investigated in future studies.

NK cells in SLE exhibit an impairment with cytotoxic function. Increasing studies have suggested that restoration of NK cell cytotoxic function by regulating SLAMF7 and CD38 can promote plasma cell deletion and contribute to SLE improvement [35]. Our data has implicated a declined level of NK cells in SLE patients, which is consistent with findings in a previous study [32]. However, no significant influence of Belimumab on NK cells is found in this study. Increasing evidence has demonstrated the critical role of NK cells in SLE, whereas current data are not sufficient enough to support their altering effects in SLE pathogenesis. Apart from NK cells, macrophages play pivotal roles in SLE by regulating inflammatory and immune responses. When stimulated by various inflammatory stimuli, mononuclear macrophages can polarize to pro-inflammatory M1 cells, while anti-inflammatory and immunosuppressive M2 cells are significantly inhibited [36]. To the best of our knowledge, imbalance of macrophages polarization and deficiency of phagocytosis and clearance for apoptotic cells are attributed to SLE pathogenesis [37, 38]. It has been well established that TLR2/1 agonist PAM3 can improve the outcome of SLE by inducing monocytes polarize to M2 phenotype in NZB/W lupus model [39], which suggests the pivotal role of M1/M2 bias in maintaining immune microenvironmental balance in SLE. However, we fail to elucidate the effect of Belimumab on the polarization of monocytes in peripheral blood in SLE patients, probably due to small sample size and insufficient available data in the present study.

Lower level of complement factors is commonly found due to its excessive consumption in SLE patients. A rise of complement factors is observed merely in self-paired SLE patients after treatment by Belimumab. Many studies have implicated platelets not only participate in primary hemostasis but contribute to leukocyte recruitment and host defense against sterile inflammation as well as exogenous pathogens. The interaction between platelets and leukocytes must be tightly regulated to avoid excessive inflammation and final tissue and organ damages in SLE [40, 41]. Overactivated B cells and abundant autoantibodies lead to an excessive destruction of platelets in SLE. An interesting phenomenon in our study is that significantly increased platelets is observed among SLE patients treated by Belimumab. Accordingly, our research has shown the potential therapeutic effect of Belimumab on thrombocytopenia in SLE, suggesting the inhibitory effect of Belimumab on autoantibodies production. Nevertheless, the potential mechanism warrants to be elucidated in the future.

The heterogeneity of lymphocytes and monocytes has offered new insight to the development and progression of SLE. Current evidence for the pathogenic role of B cells in SLE has emphasized that Belimumab may be an effective therapeutic strategy for SLE. Belimumab is essential for blocking B cell receptor (BCR) by combining with BAFF. Recent evidence provides insights into the mechanisms by which BAFF interact with BCR and TCR to regulate B cell survival and mobilization, which supports the value of targeted inhibition of B cells in SLE. Some previous clinical

trials have confirmed the efficacy and safety of Belimumab in treating SLE [42, 43]. However, little is known about the effect of Belimumab on immune cells differentiation and functions in SLE. We have demonstrated the significant heterogeneity of immune cells in peripheral blood samples from SLE patients, including those patients receiving Belimumab treatment. The most important thing is further research is needed particularly regarding the molecular mechanism of Belimumab in regulating inflammation and autoimmunity.

Findings in our study should be interpreted with caution due to several study limitations. Firstly, the main limitation of this study is the relatively small sample size. As mentioned above, we cannot elucidate the accurate effect of Belimumab on M1/M2 balance in SLE because of limited sample size and shorter observation time. Secondly, the difference between individuals is a significant confounding factor that cannot be ignored in this study. Thirdly, the modifying effects and regulatory mechanisms of Belimumab on immune cells, T cell, B cell, and monocytes in particular, warrant to be investigated in future studies.

Conclusion

In summary, the heterogeneity of lymphocytes and monocytes reveals their pivotal roles in SLE immune microenvironment. Anti-BAFF treatment is a promising strategy for SLE patients, particularly those difficult to obtain clinical relief. Belimumab not only exerts its inhibitory effect on B cells but may regulate other immunes, including T cell and monocytes. Last but not the least, more comprehensive research is warranted to elucidate the regulatory mechanism of Belimumab on immune cells. In addition, more clinical experiments and follow-up studies are still needed to estimate the efficacy and safety of anti-BAFF reagent, Belimumab.

Abbreviations

HC: health control; SLE: Systemic lupus erythematosus; IFN: interferons; TLRs: Tolllike receptors; BAFF: B-cell activating factor; BLyS: B-lymphocyte stimulator; ESR: erythrocyte sedimentation rate; C3: serum concentrations of complement factors 3; C4: serum concentrations of complement factors 4; WBC: white blood cell count; RBC: erythrocyte count; PLT: platelet count; mALB: micro-albminuria; ANA: antinuclear antibody; MFI: mean fluorescence intensity; SD: standard deviation; BCR: B cell receptor

Declarations

Acknowledgements

Author contributions

Funding

Availability of data and materials

Data and materials available for this study would require further approval upon request from the corresponding author.

Ethics approval and consent to participate

This work received approval from the Institutional Ethics Committee of the First Affiliated Hospital of Weifang Medical University. All subjects had read and signed a written informed consent before enrollment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Demographic and clinical data of Study Samples

Feature	HC	Belimumab-treated SLE	Belimumab-untreated SLE
Sample size	92	26	140
Age (year)	31.73±7.74	32.15±11.14	36.76±12.18 *
Male	2	4	10
Female	90	22	130

Data are presented as mean ± SD. *▯ compared with HC group, $P < 0.05$▯ ▲▯compared with that Belimumab treated group, $P < 0.05$ (Student's t-test).

Table 2 Clinical data from SLE patients

	Belimumab-untreated (n=140)	Belimumab-treated (n=26)	Pre-treatment (n=17)	Post-treatment (n=17)
ESR (mm/h)	24.8±22.97	17.04±10.73	24.13±13.98	18.53±10.76
C3 (g/L)	1.38±4.82	0.75±0.23	0.61±0.26	0.77±0.21 ▲
C4 (g/L)	0.16±0.09	0.18±0.1	0.13±0.09	0.18±0.09 ▲
WBC (×10^9/L)	8.15±23.92	5.99±2.35	6.71±2.11	6.02±2.35
RBC (×10^9/L)	7.0±32.48	4.4±0.57	4.3±0.7	4.41±0.67
PLT (×10^9/L)	215±75.76	247.5±54.8 *	213.7±63.45	240.3±67.05 ▲
mALB (mg/L）	453.5±1088	512.0±915.7	1410±1611	686.0±1149 ▲
ANA (au/mL)	319.5±192.4	306.3±293.8	329.2±167.8	392.4±181.9
Anti-dsDNA antibody (u/MI)	101.3±97.92	148.6±128.3	144.1±123.1	164.5±147.0

Data are presented as mean ± SD. ESR, erythrocyte sedimentation rate (0-20mm/h); C3, serum concentrations of complement factors 3 (0.79-1.52g/L); C4，serum concentrations of complement factors 4 （0.16-0.38g/L）; WBC，white blood cell count （4.0-10.0×10^9/L）; RBC，erythrocyte count （3.5-5×10^12/L）; PLT，platelet count （100.00-300.00×10^9/L）; mALB，micro-albminuria （0-30mg/L）; ANA，antinuclear antibody （0-32au/mL）; Anti-dsDNA antibody（0-25u/MI）. *，compared with untreated group, P < 0.05，▲，compared with that pre-treated group, P < 0.05 (Student's t-test).

Table 3. Distribution of lymphocyte subsets in three study groups.

	HC		SLE untreated with Belimumab		SLE treated with Belimumab	
	Percentage (%)	Absolute count	Percentage (%)	Absolute count	Percentage (%)	Absolute count
CD3$^+$	71.94±6.72	1384±418.3	79.11±9.74 *	1058±741.8 *	86.2±7.3 *▲	1279±610.3
CD3$^+$CD4$^+$	37.67±6.81	721.7±233.4	35.14±8.79 *	484.4±410.9 *	37.2±10.48	555.2±330.7 *
CD3$^+$CD8$^+$	29.73±5.83	574.5±204.2	40.84±11.48 *	536±358.2	45.55±12.82 *	667.3±363.8
CD3$^+$CD4$^+$/ CD3$^+$CD8$^+$	1.34±0.47	——	0.97±0.48 *	——	0.92±0.43 *	——
CD19$^+$	11.41±3.17	218.8±87.31	11.06±7.82	141.6±128.4 *	3.85±3.41 *▲	53.5±53.59 *▲
CD16$^+$CD56$^+$	15.41±6.76	294.6±153.9	8.57±6.21*	110.6±158.3 *	8.06±7.44 *	113.8±133.9 *

Data are presented as mean ± SD. *, compared with HC group, $P < 0.05$; ▲compared with SLE untreated group, $P < 0.05$ (Student's t-test).

Table 4. Distribution of monocyte subsets in HC, SLE untreated and treated with Belimumab.

	CD14$^+$HLA-DR$^+$ monocyte		CD14$^+$CD206$^+$ monocyte		CD14$^+$CD163$^+$ monocyte	
	Percentage (%)	MFI	Percentage (%)	MFI	Percentage (%)	MFI
HC (n=14）	97.80±3.03	11365±2533	22.06±11.29	4330±2099	98.34±2.52	5638±1832
SLE untreated with Belimumab (n=16)	95.68±4.28	20457±27742	8.79±14.89 *	14693±23161	82.95±9.31*	3228±1909 *
SLE treated with Belimumab (n=16)	95.97±3.63	12167±3235	5.14±9.66 *	13310±17834	85.24±6.2 *	3127±1717 *

Data are presented as mean ± SD. *, compared with HC group, $P < 0.05$ (Student's t-test).

Table 5. Distribution of Lymphocyte subsets in patients with SLE before and after Belimumab treatment.

	CD3$^+$	CD3$^+$CD4$^+$	CD3$^+$CD8$^+$	CD3$^+$CD4$^+$/ CD3$^+$CD8$^+$	CD19$^+$	CD16$^+$CD56$^+$
SLE pre-treated with Belimumab (n=17)						
Percentage (%)	80.2 ± 7.8	34.11±10.82	43.11 ± 10.72	0.89 ± 0.5	8.61 ± 4.6	8.8 ± 7.1
Absolute value	1100±825.4	481.0±386.3	578.2 ± 437.3	—	133.4 ± 119.2	110.8 ± 118.1
SLE post-treated with Belimumab (n=17)						
Percentage (%)	85.68 ± 8.76▲	35.51±11.67	46.63 ± 14.85	0.88 ± 0.47	4.07 ± 3.51▲	8.08 ± 7.32
Absolute value	1295±677.1	552.4 ± 396.5	684.6 ± 381.1	—	62.47 ± 66.47▲	167.8 ± 232.1

Data are presented as mean ± SD. ▲ compared with SLE pre-treated with Belimumab group, $P < 0.05$ (self-paired t-test).
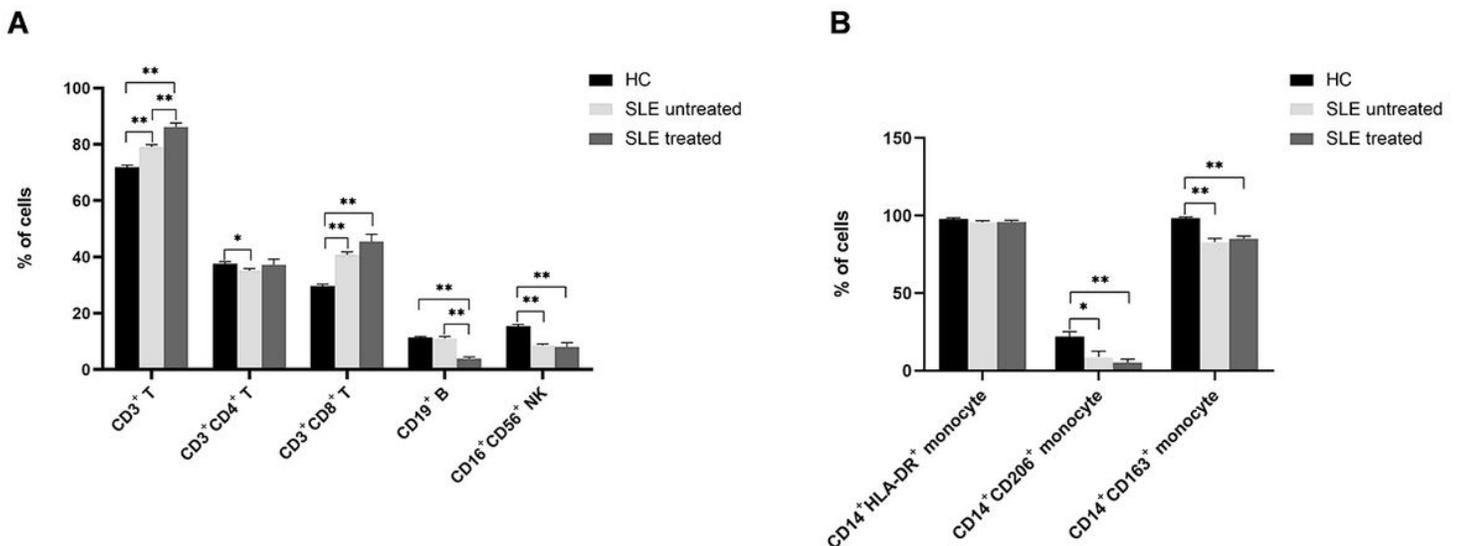
Figures

Figure 1

Frequencies of immune cells in peripheral blood samples. SLE patients have higher level of CD3+ T cells and CD3+CD8+ T cells than HCs, while lower level of CD3+CD4+ T cells, CD16+CD56+ NK cells, CD14+CD206+ monocytes and CD14+CD163+ monocytes. Belimumab treatment led to decreased CD19+ B cells and CD3+ T cell. *, P<0.05; **, P<0.01.
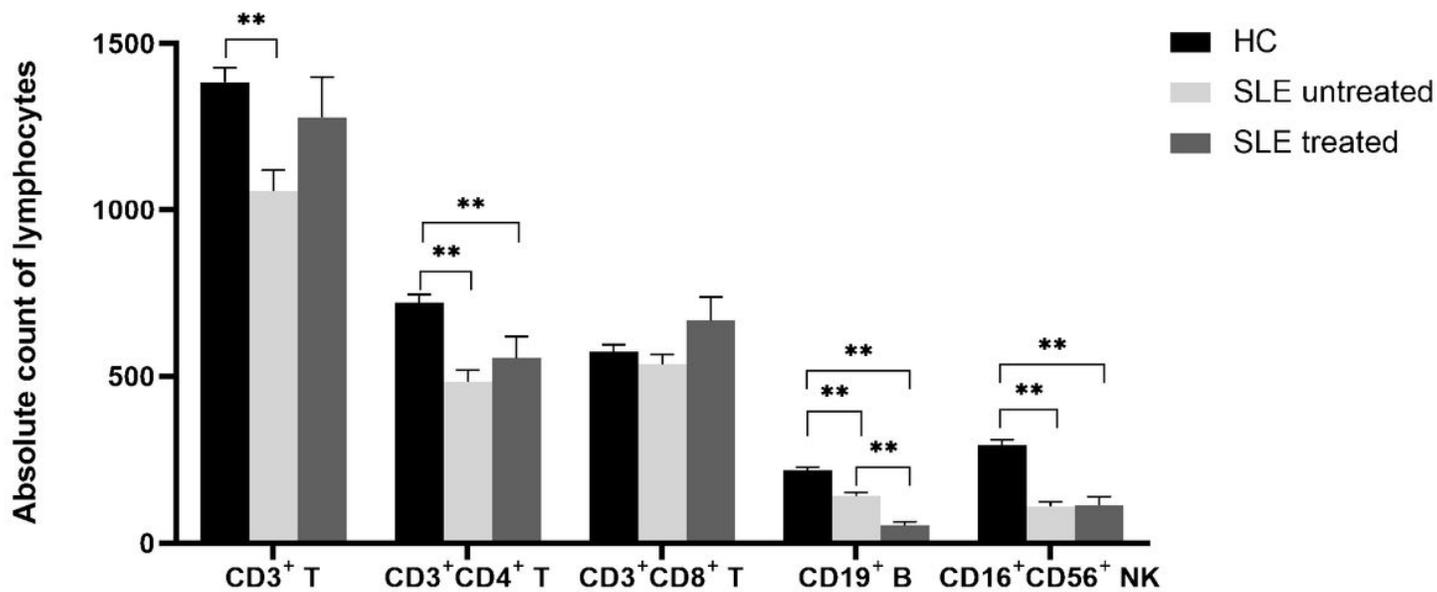
Figure 2

Absolute count of lymphocytes in peripheral blood samples. CD3+ T cells、CD3+CD4+ T cells、CD19+ B cells and CD16+CD56+ NK cells in patients were significantly lower than HCs. A significant decrease of CD19+ B cells was found in SLE patients in response to Belimumab treatment. *, $P<0.05$; **, $P<0.01$.
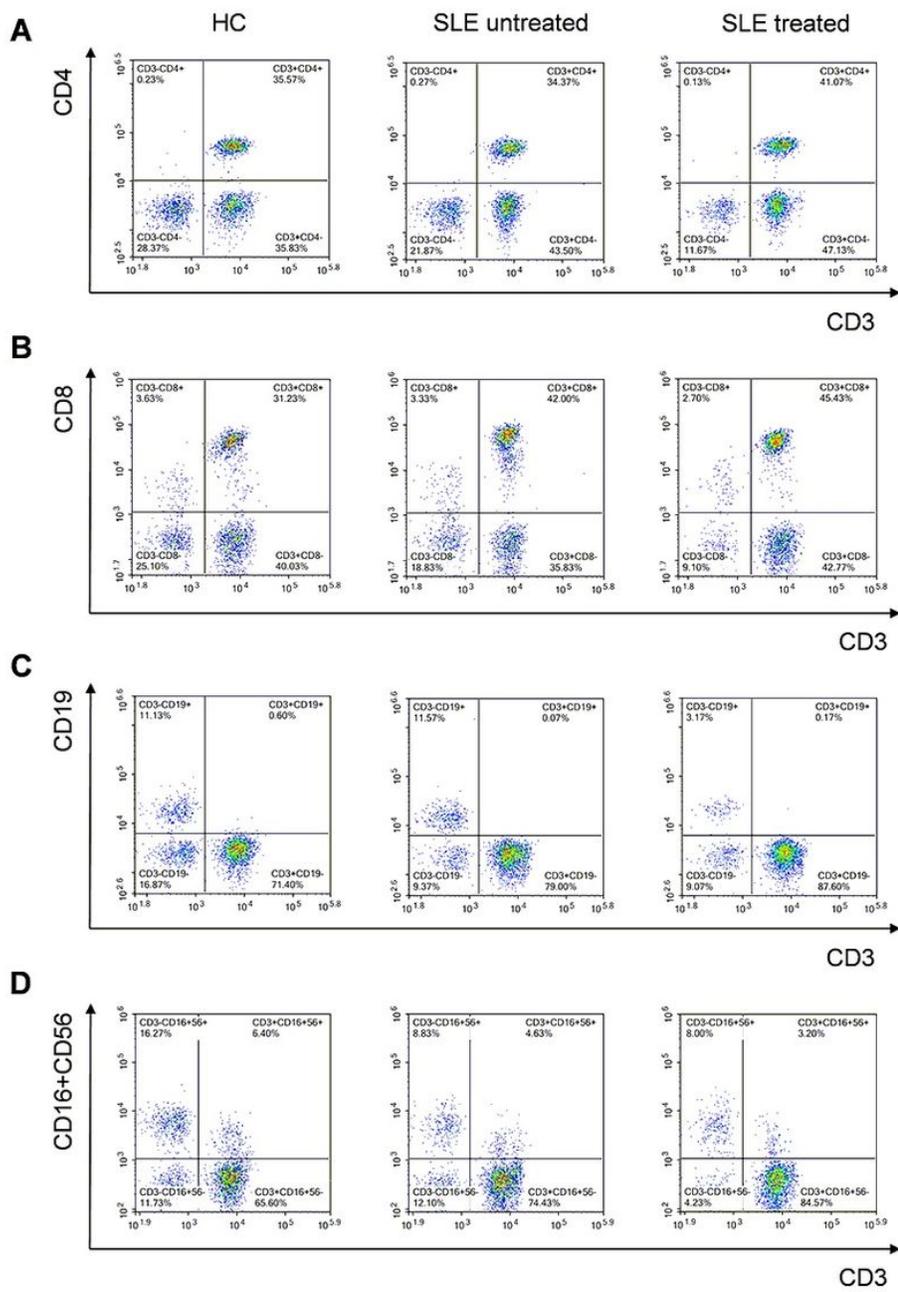
Figure 3

Representative pictures for flow cytometry: CD3+CD4+ T cell, CD3+CD8+ T cell, CD19+ B cell and CD16+CD56+ NK cell between groups.
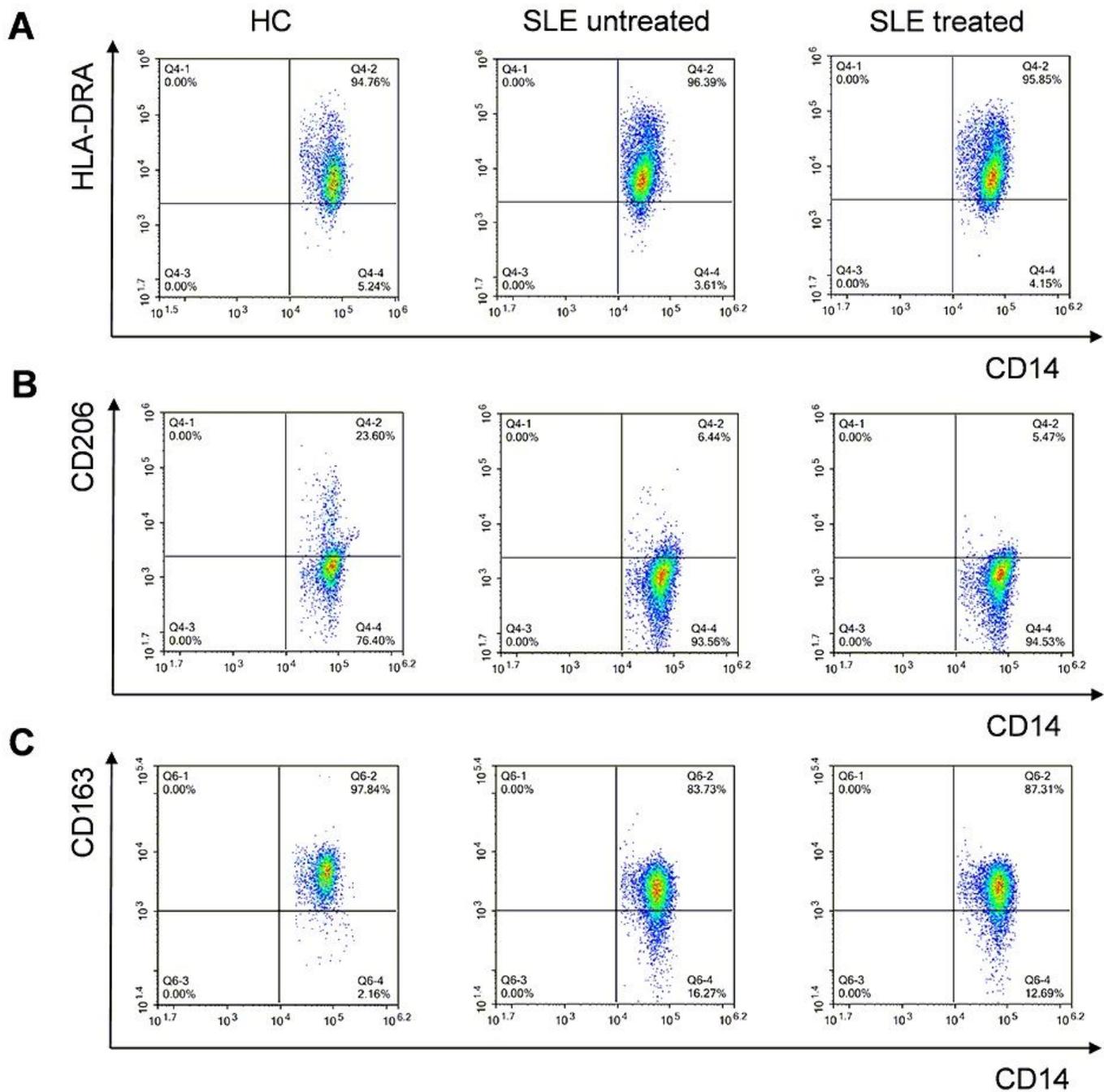
Figure 4

Representative pictures for flow cytometry: CD14+HLA-DR+ monocyte, CD14+CD206+ monocyte and CD14+CD163+ monocyte between groups.
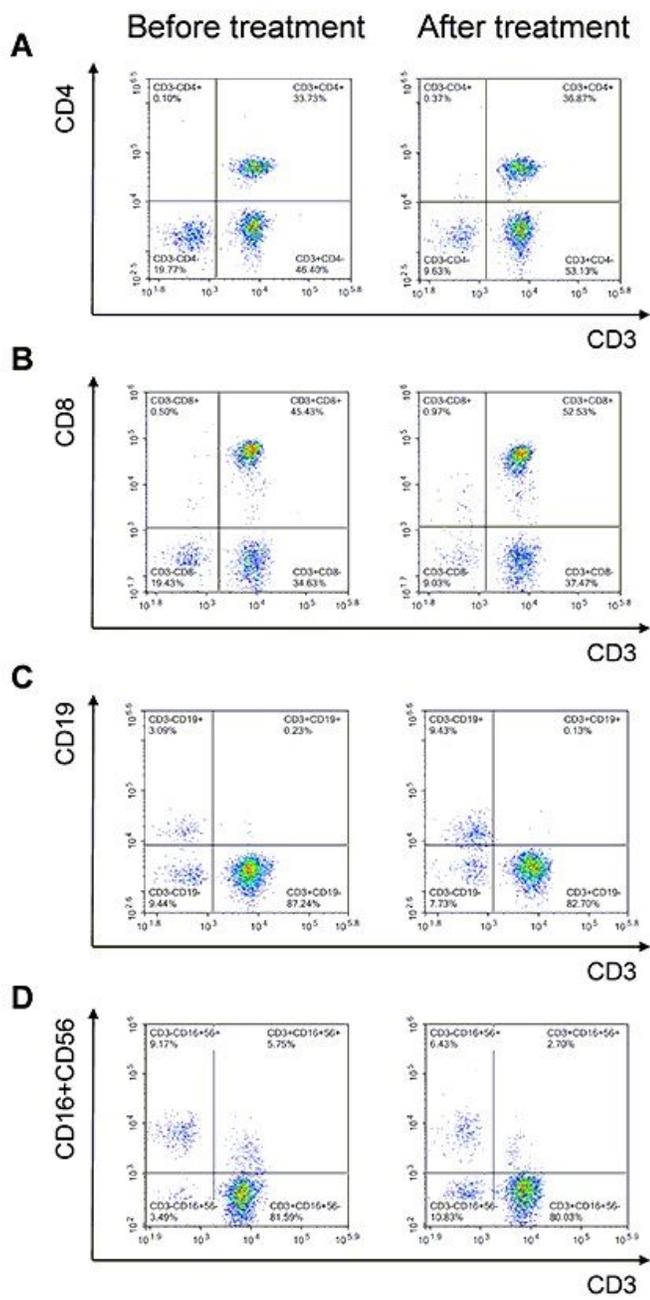
Figure 5

Representative pictures for flow cytometry: CD3+CD4+ T cell, CD3+CD8+ T cell, CD19+ B cell and CD16+CD56+ NK cell between groups.