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IL-10-producing regulatory B cells are decreased in patients with psoriasis

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ABSTRACT

Backgrounds: Interleukin (IL)-10-producing regulatory B cells (B10 cells) have been shown to ameliorate psoriasis in mice. Human B10 progenitor cells are characterized as $CD19^+CD24^{hi}CD38^{hi}$ B cells that exert their regulatory functions via the production of IL-10. However, the role of B10 cells in the pathogenesis of psoriasis remains unclear.
Objectives: We examined B10 cells in patients with psoriasis and healthy controls.
Methods: Peripheral blood mononuclear cells were isolated from psoriasis patients without a history of receiving any immunosuppressants during the 6-month period before enrollment in the study. Using flow cytometry, we determined the frequencies of blood B cell subsets, B10 progenitor cells, and B10 cells for 31 patients with psoriasis and 26 healthy controls.
Results: Both psoriasis patients and healthy controls showed similar frequencies of total B cells, IgD^+CD27^- naïve B cells, and IgD^-CD27^+ memory B cells. However, the frequency of $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells was significantly higher in patients with psoriasis than in the healthy controls. In contrast, the frequency of B10 cells in patients with psoriasis was significantly lower than that in healthy controls. Furthermore, treatment with immunosuppressants resulted in a decrease in B10 progenitor cells and an increase in B10 cells.
Conclusion: B10 progenitor cells were increased, while IL-10-producing regulatory B10 cells were decreased in patients with psoriasis, suggesting that B10 cells may be functionally impaired in patients with psoriasis.
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1. Introduction

Psoriasis is a chronic inflammatory skin disorder characterized with an estimated prevalence of about 2% of the general population [1]. Recent studies on psoriasis have shown that both T helper (Th) 17 and Th1 cells play critical roles in the pathogenesis of the disease, while the role of B cells remains unclear.

Because of their key contributions to humoral immune responses involving the secretion of antibodies, B cells are generally considered pathogenic in the majority of autoimmune diseases [2]. However, recent evidence indicates that B cells and specific B cell subsets can negatively regulate immune responses in mice, suggesting the existence of regulatory B cells [3]. Subsequently, a specific subset of regulatory B cells with the $CD1d^{hi}CD5^+$ phenotype was identified in mice [4]. This subset has been shown to play an important inhibitory role in contact hypersensitivity responses [4]; autoimmune encephalomyelitis in an experimental model of multiple sclerosis [5]; and a dextran sulfate sodium-induced model of ulcerative colitis, in an interleukin (IL)-10-dependent manner [6]. Considering the possibility that multiple regulatory B cell subsets may exist, the cells of this subset that appear to produce only IL-10 and are responsible for most of the B-cell IL-10 production were defined as B10 cells to distinguish it from other possible regulatory B cell subsets [4,7].

The progenitors of human blood B10 cells were characterized as $CD19^+CD24^{hi}CD38^{hi}$ B cells known as transitional B cells [8], although other candidates such as $CD19^+CD24^{hi}CD27^+$ [9] and $CD19^+CD27^+$ [10] have also been proposed. Human

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$CD19^+CD24^{hi}CD38^{hi}$ B cells exhibit regulatory capacity and are reported to suppress Th1 and Th17 differentiation throughout the process of IL-10 production [11]. B10 cell abnormalities have been reported in cases of rheumatoid arthritis [12], systemic lupus erythematosus [13], pemphigus [14], sarcoidosis [15], and anti-neutrophil cytoplasm antibody-associated with vasculitis [16]. In addition, B10 cells have been shown to play regulatory roles in systemic lupus erythematosus [13], rheumatoid arthritis [12], and pemphigus [14].

B10 cells have been shown to suppress the imiquimod-induced psoriasis-like skin inflammation in mice [17]. In humans, the severity of psoriasis was reduced by treatment with recombinant human IL-10 [18,19]. Furthermore, B cell depletion in humans, achieved by administering chimeric human anti-CD20 monoclonal antibody (mAb) rituximab, leads to the development of psoriasis in some cases [20,21]. These factors suggest that both B cells and IL-10 play important inhibitory roles in the pathogenesis of psoriasis. Therefore, in this study, we examined the pathogenic roles of B10 cells in patients with psoriasis.

2. Materials and methods

2.1. Subjects

The study group comprised 26 healthy controls and 31 patients with psoriasis vulgaris. Eligible patients were those who had been diagnosed with psoriasis based on clinical features or histopathological findings and had not received any immunosuppressant in the previous 6 months. The Psoriasis Area and Severity Index (PASI) was used to assess cutaneous disease severity at the site of skin involvement. Written informed consent was obtained from all the enrolled patients and healthy controls. The study protocol was approved by the ethics committee of The Jikei University and was in accordance with the Declaration of Helsinki.

2.2. Cell isolation and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples, by performing gradient centrifugation using Lymphoprep (Axis-Shield PoC As, Oslo, Norway). Then, the isolated PBMCs were resuspended (2×10^6 cells/ml) in complete medium (RPMI 1640 (Sigma–Aldrich, St. Louis, MO) containing 10% fetal calf serum (Sigma–Aldrich), 110 U/ml penicillin (Gibco, Auckland, New Zealand), 110 μg/ml streptomycin (Gibco), and 0.3212 mg/ml L-glutamine (Gibco)). The anti-human fluorochrome-conjugated antibodies used included FITC-conjugated CD19 (BioLegend, San Diego, CA), PerCP-conjugated CD27 (BioLegend), PE/Cy7-conjugated CD24 (BioLegend), Pacific Blue-conjugated CD95 (BioLegend), Pacific Blue-conjugated CD86 (BioLegend), Pacific Blue-conjugated CD80 (EXIBO Pharma, Vestec u Prahy, Czech Republic), and PE-conjugated IgD mAbs (BD Biosciences, San Diego, CA). Cells were analyzed using MACSQuant

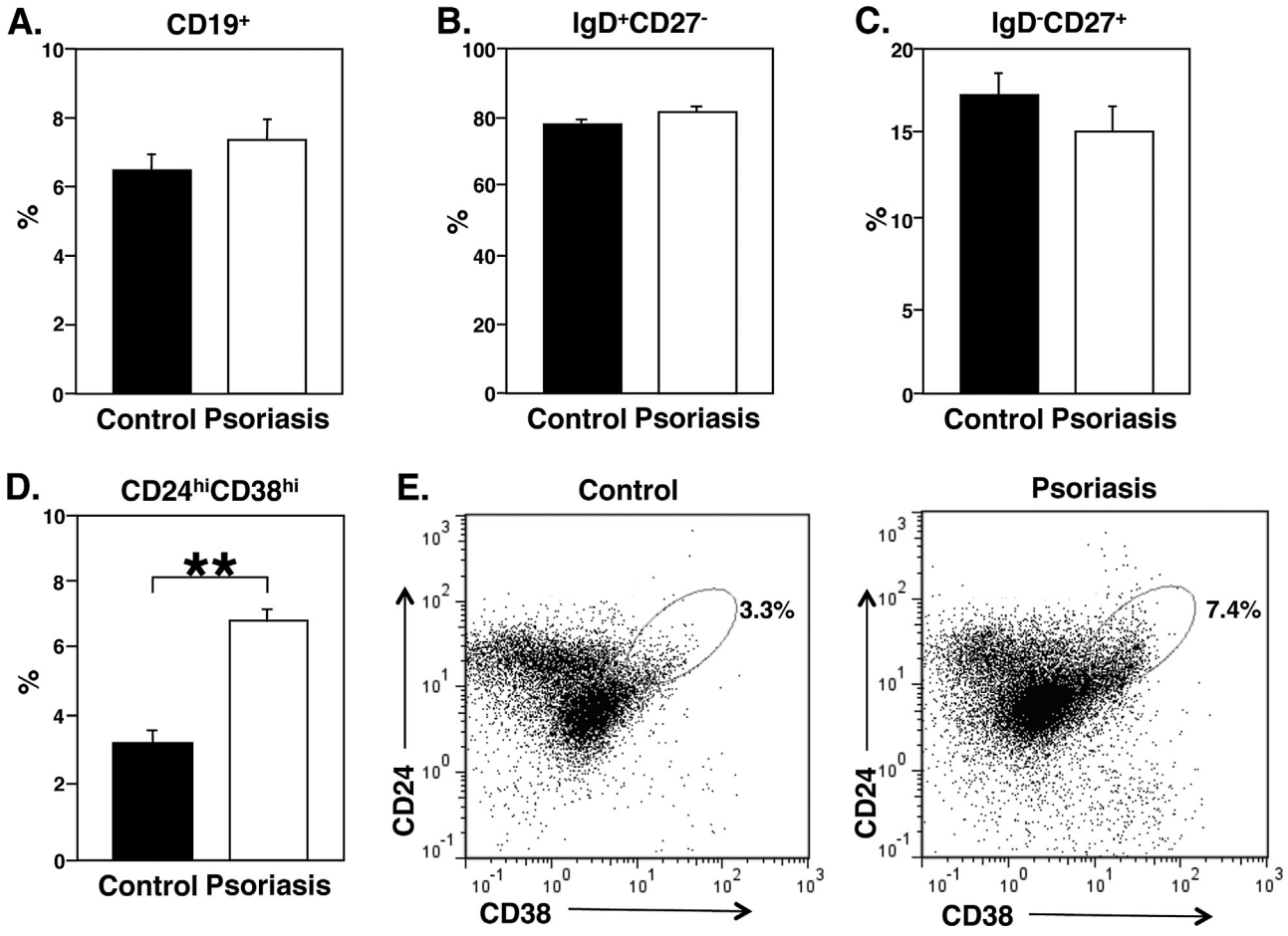

Fig. 1. The frequencies of B cell subsets in peripheral blood in patients with psoriasis and healthy controls. The frequencies of $CD19^+$ total B cells (A), $CD19^+IgD^+CD27^-$-naïve B cells (B), $CD19^+IgD^-CD27^+$ memory B cells, (C) and $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells (D) in patients with psoriasis and in healthy controls were determined by flow cytometric analysis. $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells were determined using the gates shown in (E). Samples of 31 patients with psoriasis and 26 healthy controls were analyzed. Bar graphs indicate mean (±SEM) percentages of B cells and B-cell subsets. Significant differences are noted between the sample means: $^{**}P < 0.01$.

(Miltenyi Biotec, Auburn, CA). All analyses were performed using fresh blood samples.

2.3. Assessment of IL-10-producing B Cells

Intracellular IL-10 analysis by flow cytometry was performed as described previously [9]. Briefly, PBMCs were resuspended (2×10^6 cells/ml) in complete medium in the presence of CpG (Toll-like receptor 9 ligand, ODN 2006 Type B, 10 μg/ml; InvivoGen, San Diego, CA) and CD40 ligand (CD40L, 1 μg/ml; R&D Systems, Minneapolis, MN) in 24-well flat bottom plates for 48 h at 37 °C. Phorbol 12-myristate 13-acetate (PMA, 50 ng/ml; Sigma–Aldrich), ionomycin (1 μg/ml, Sigma–Aldrich), and brefeldin A (5 μg/ml, BioLegend) were added for the last 5 h. After membrane staining, the cells were fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) and stained with anti-human PE-conjugated IL-10 mAb (BioLegend). The extent of background staining was determined using unreactive isotype-matched control mAb (eBioscience, San Diego, CA) with gates positioned to exclude ≥98% of unreactive cells.

2.4. Measurement of circulating IL-10 and B-cell-activating factor (BAFF)

IL-10 and B-cell-activating factor (BAFF) levels were measured in serum samples obtained from patients with psoriasis and healthy controls by using the specific ELISA kits (R&D Systems.) according to the manufacturer's instructions.

2.5. Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U*test for two-group comparison, the Kruskal–Wallis test and the Bonferroni test for multiple comparisons, and Wilcoxon matched-pairs signed-rank test for paired comparison. All data are shown as mean ± SEM values. *P* values less than 0.05 were considered significant.

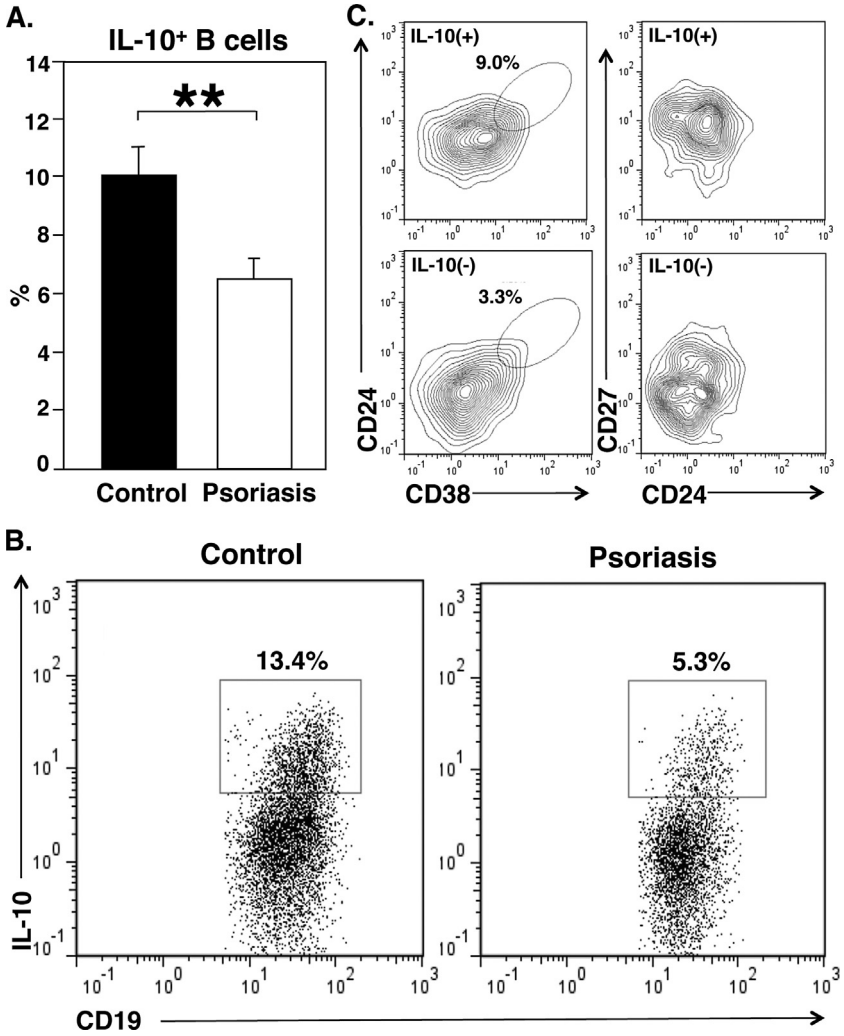

Fig. 2. The frequency and phenotypes of B10 cells in peripheral blood. The frequency of IL-10-producing B cells in peripheral blood after stimulation for 48 h with CpG and CD40L and for the last 5 h with PMA, ionomycin, and brefeldin A. The frequencies of B10 cells in patients with psoriasis and in healthy controls (A). The representative results of B10 cells in patients with psoriasis and in healthy controls (B). Phenotypes of B10 cells in peripheral blood (C). Distribution of B10 cells within B cell subsets defined by CD24/CD38 and CD24/C27 expression in patients with psoriasis and in healthy controls. Samples of 18 patients with psoriasis and 13 healthy controls were analyzed. Bar graphs indicate mean (±SEM) percentages of B10 cells. Significant differences between sample means are indicated: $^{**}P < 0.01$.

3. Results

3.1. B10 progenitor cells are increased in patients with psoriasis

To evaluate the possible changes in B-cell populations in patients with psoriasis, we compared the frequencies of $CD19^+$ total B cells, $CD19^+IgD^+CD27^-$ naïve B cells, $CD19^+IgD^-CD27^+$ memory B cells, and $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells in patients with psoriasis and healthy controls. Samples of 31 patients with psoriasis and 26 healthy controls were examined by flow cytometry. Both patients with psoriasis and healthy controls had similar frequencies of $CD19^+$ B cells (Fig. 1A), $CD19^+IgD^+CD27^-$ naïve B cells (Fig. 1B), and $CD19^+IgD^-CD27^+$ memory B cells (Fig. 1C). Furthermore, the frequencies of $CD19^+$B cells, naïve B cells, and memory B cells were comparable between patients with psoriasis and healthy controls. In contrast, the frequency of $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells in patients with psoriasis was 2.2-fold higher than that in healthy controls ($7.0 \pm 0.5\%$ vs. $3.2 \pm 0.3\%$; $P < 0.01$) (Fig. 1D). Examples of gates used for $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells in a healthy control and a patient with psoriasis are shown in Fig. 1E. Thus, B10 progenitor cells were increased and frequencies of other B-cell subsets were normal in patients with psoriasis.

3.2. B10 cells are decreased in patients with psoriasis

To assess whether the ability to produce IL-10 by B cells remains intact in paints with psoriasis, PBMCs isolated from patients with psoriasis and healthy controls were cultured with CpG and CD40L for 48 h, with the addition of PMA, ionomycin, and brefeldin A during the last 5 h. After stimulation, the extent of intracellular staining of IL-10 was assessed. Samples of 18 patients with psoriasis and 13 healthy controls were examined. The frequency of B10 cells in patients with psoriasis was significantly lower than that in healthy controls (35% decrease; $6.6 \pm 0.7\%$ vs. $10 \pm 1.1\%$, $P < 0.01$; Fig. 2A and B). To confirm whether $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells produce IL-10, we next examined the levels of CD24 and CD38 expression in IL-10-producing B cells from peripheral blood. CD24 and CD38 were expressed at higher levels in $IL\text{-}10^+$ than in $IL\text{-}10^-$ B cells (Fig. 2C). Because other phenotypes of B10 cells such as $CD19^+CD24^{hi}CD27^+$ [9] and $CD19^+CD27^+$ [10] have been also proposed, we also examined the distributions of $IL\text{-}10^+$ B cells by CD24/CD27 expression (Fig. 2C). Although $IL\text{-}10^+$ B cells tended to show higher CD27 expression than $IL\text{-}10^-$ B cells as previously reported [9], we adopted $CD19^+CD24^{hi}CD38^{hi}$ phenotype as B10 progenitor cells in the current study.

3.3. Frequencies of B10 progenitor cells and B10 cells do not correlate with disease severity

We examined whether the severity of psoriasis correlated with the frequencies of $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells B cells and B10 cells in peripheral blood. We used PASI scores to assess disease severity in the patients with psoriasis. Both the frequencies of B10 progenitor cells and B10 cells did not correlate with PASI scores (Fig. 3A and B).

3.4. Serum IL-10 levels in patients with psoriasis

The frequency of B10 cells in patients with psoriasis was decreased compared with healthy controls. Therefore, we next evaluated the serum IL-10 levels in patients with psoriasis and healthy controls. Samples of 19 patients with psoriasis and 17 healthy controls were examined. Serum IL-10 levels in patients with psoriasis and healthy controls did not differ significantly (0.46 ± 0.07 pg/ml vs. 0.47 ± 0.17 pg/ml; Fig. 4A).

3.5. Expressions of CD80, CD86, and CD95 on total B cells and B10 progenitor cells in patients with psoriasis

To determine whether there was difference in the activation status of B cells between patients with psoriasis and healthy controls, we investigated the expression of various activation markers, including CD80, CD86, and CD95 by immunofluorescence staining with flow cytometry analysis. Samples of six patients with psoriasis and 6 healthy controls were examined. The mean expression levels of CD80, CD86, and CD95 on total B cells in patients with psoriasis and healthy controls were comparable

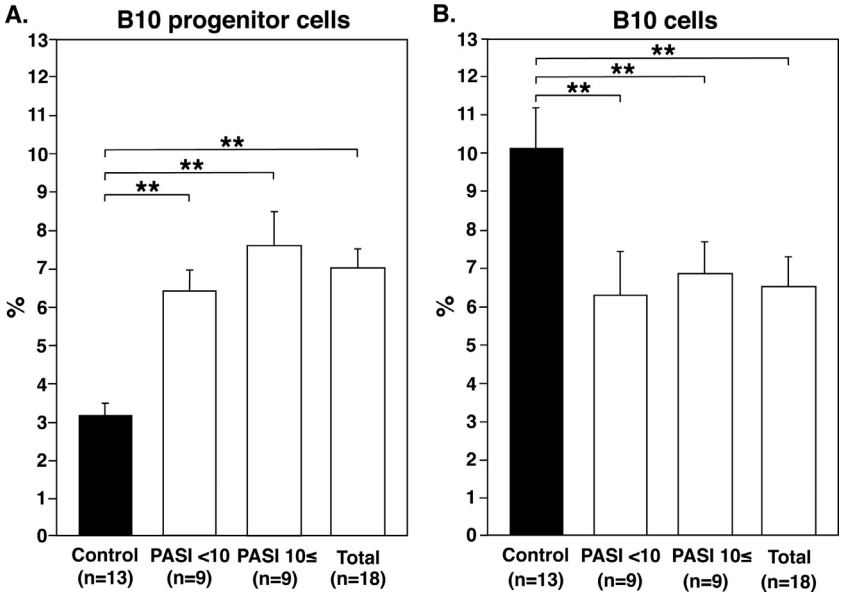

Fig. 3. The frequencies of $CD19^+CD24^{hi}CD38^{hi}$ B10 progenitor cells and B10 cells in peripheral blood. Patients with psoriasis were classified into 2 groups on the basis of their PASI scores. The frequencies of B10 progenitor cells (A) in peripheral blood were examined in 31 patients with psoriasis and 26 healthy controls. The frequencies of B10 cells (B) in peripheral blood after stimulation for 48 h with CpG and CD40L, and for the last 5 h with PMA, ionomycin, and brefeldin A, were determined in 18 patients with psoriasis and 13 healthy controls. Bar graphs indicate the mean (±SEM) percentages of B cell subsets. Significant differences between sample means are indicated: $^{**}P < 0.01$.

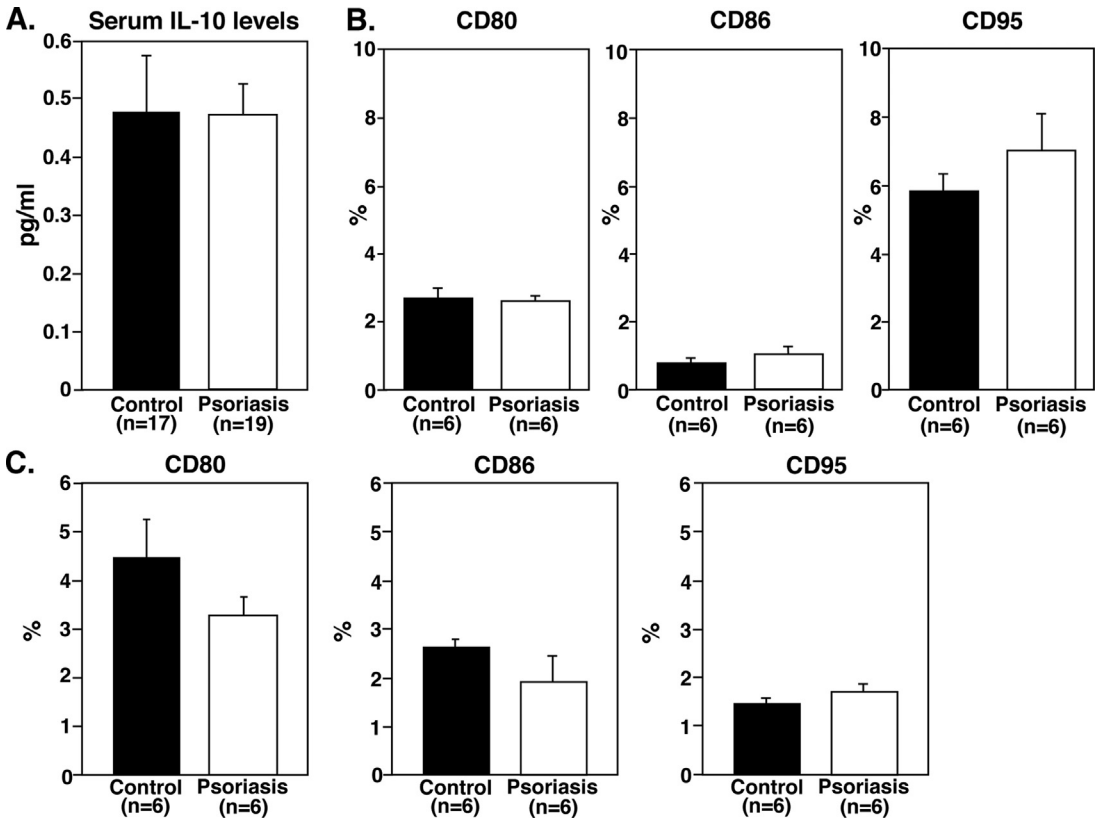

Fig. 4. Serum IL-10 levels in patients with psoriasis and healthy controls (A). Expression levels of CD80, CD86, and CD95 on B cells (B) (C). Nineteen patients with psoriasis and 17 healthy controls were examined. Bar graphs indicate mean (±SEM) serum IL-10 levels (A). We investigated expression of activation markers, CD80, CD86, and CD95 by immunofluorescence staining with flow cytometry analysis. Samples of 6 patients with psoriasis and 6 healthy controls were analyzed. Bar graphs indicate mean (±SEM) expression of activation markers on total B cells (B) and on $CD24^{hi}CD38^{hi}$ B10 progenitor cells (C).

(Fig. 4B). Since the frequency of B10 progenitor cells was significantly higher in patients with psoriasis than in healthy controls, we sought to elucidate the peculiar activation status of B10 progenitor cells in patients with psoriasis. Accordingly, we detected the expression levels of CD80, CD86, and CD95 on $CD24^{hi}CD38^{hi}$ B10 progenitor cells in patients with psoriasis and healthy controls. The mean expression levels of CD80, CD86, and CD95 on B10 progenitor cells in patients with psoriasis and healthy controls did not show any significant difference (Fig. 4C). Therefore, patients with psoriasis and healthy controls had comparable expression of CD80, CD86, and CD95 on both total B cells and B10 progenitor cells.

3.6. Influence of therapeutic intervention on B10 progenitor cells and B10 cells

To determine whether the frequencies of B10 and B10 progenitor cells are altered over time, 11 patients with psoriasis were followed up after initiation of immunosuppressant treatment. The mean follow-up period was 5.2 ± 1.0 months (range, 1–10 months). After the initial evaluation, 3, 7, and 1 patient were treated with infliximab, ustekinumab, and cyclosporine, respectively. At baseline, mean PASI scores and the frequencies of B10 progenitor cells and B10 cells were 13.4 ± 2.9, 7.5 ± 1.0%, and 5.8 ± 0.8%, respectively (Fig. 5); after initiating treatment with immunosuppressants, the corresponding values were 2.7 ± 0.9, 3.6 ± 0.5%, and 10.6 ± 0.9%. The frequency of B10 progenitor cells decreased by 52% ($P < 0.01$), while the frequency of B10 cells was increased by 84% after immunosuppressant treatments ($P < 0.01$). Overall, 9 of the 11 patients treated with immunosuppressant treatment exhibited at least a 30% increase in frequencies of B10 cells compared with the baseline level. Consequently, immunosuppressant treatments reduced B10 progenitor cells, while it expanded B10 cells in patients with psoriasis. To confirm if topical treatment did not affect the frequencies of B10 cells, we compared the frequencies of B10 cells between patients treated with immunosuppressants and those treated with topical therapy alone. The frequency of B10 cells in the patients treated with immunosuppressants was significantly higher than in those treated with topical therapy alone ($P < 0.01$; Fig. 6). Thus, therapeutic intervention with immunosuppressants but not topical treatment might increase the frequency of B10 cells.

3.7. Increased serum BAFF levels in patients with psoriasis and influence of therapeutic intervention on serum BAFF levels

BAFF is involved in B-cell development, survival, and immunoglobulin production [22]. We next compared the serum BAFF levels in healthy controls and patients with psoriasis. Samples collected from 32 healthy controls and 37 patients with psoriasis were examined. Patients with psoriasis had significantly increased serum BAFF levels (1744 ± 102 pg/ml), compared with the healthy controls (1456 ± 58 pg/ml, $P < 0.01$; Fig. 7A). However, the BAFF levels did not correlate with the frequencies of B10 progenitor cells and B10 cells. We next examined the influence of treatment on serum BAFF levels. Sixteen of the patients included in the serum BAFF levels study were followed up after initiation of treatment with immunosuppressants. The mean time of follow-up from initial treatment was 4.3 ± 0.5 months (range, 2–8 months). Eleven and 5 patients were treated with infliximab and ustekinumab,

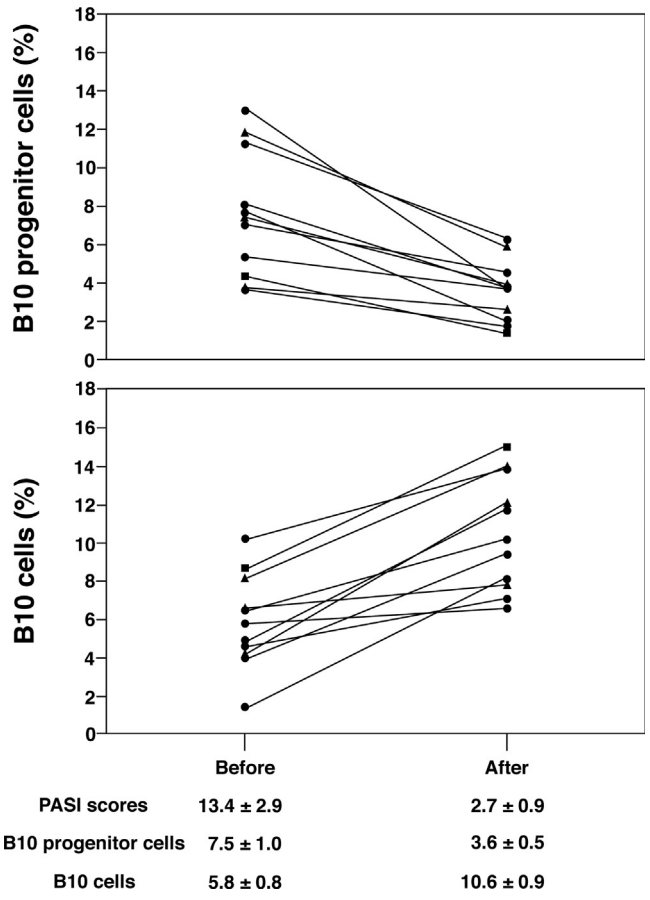

Fig. 5. Changes in $CD24^{hi}CD38^{hi}$ B10 progenitor cells and B10 cells after therapeutic intervention with immunosuppressants. Eleven of the patients included in the B10 cells study were followed up. Circles, triangles, and squares indicate patients treated with ustekinumab, infliximab, and cyclosporine, respectively. $^{**}P < 0.01$ vs. frequencies of $CD24^{hi}CD38^{hi}$ B10 progenitor cells and B10 cells at baseline. PASI scores, the frequencies of B10 progenitor cells and those of B10 cells at baseline and after treatments are shown as mean (±SEM).

respectively. At baseline, the mean PASI scores and serum BAFF levels were 14.4 ± 2.1 and 1790 ± 150 pg/ml, respectively (Fig. 7). After initiating treatment with immunosuppressants, mean PASI scores and the serum BAFF levels changed to 10.6 ± 0.9 and 1204 ± 147 pg/ml, respectively. The serum BAFF levels were decreased by 33% after starting treatment with immunosuppressants ($P < 0.01$). Overall, 13 of the 16 patients treated with immunosuppressants exhibited at least a 20% decrease in serum BAFF levels, compared with baseline levels. Thus, we noted that serum BAFF levels were increased in patients with psoriasis, and therapeutic intervention with immunosuppressants reduced the serum BAFF levels.

4. Discussion

To our knowledge, this is the first study to evaluate the abnormalities of IL-10-producing regulatory B cells, namely B10 cells, in patients with psoriasis. $CD19^{+}CD24^{hi}CD38^{hi}$ B cells have previously been identified as the progenitors of B10 cells and are known to exert their immunosuppressive capacities mainly via IL-10 secretion [8]. The findings of this study indicate that the number of circulating B10 progenitor cells in patients with psoriasis was significantly higher than that in healthy controls. On the contrary, the frequency of B10 cells was significantly lower in patients with psoriasis than that in healthy controls. These changes showed no correlation with disease severity as measured by PASI scores. Furthermore, therapeutic intervention with immunosuppressants substantially increased the frequency of B10 cells, but decreased the frequency of $CD24^{hi}CD38^{hi}$ B cells. In addition, our results showed no difference in the frequencies of $CD19^{+}$ B cells, $CD19^{+}IgD^{+}CD27^{-}$-naïve B cells, and $CD19^{+}IgD^{-}CD27^{+}$ memory B cells between patients with psoriasis and healthy controls. Therefore, B10 cells may be functionally impaired in patients with psoriasis.

The results of our study demonstrated that the frequency of B10 progenitor cells was increased, while that of B10 cells was decreased in patients with psoriasis, suggesting that there were potential abnormalities in the process of B10 cell development from B10 progenitor cells in patients with psoriasis. It is possible that the decrease in B10 cells augment the development of B10 progenitor cells as a negative feedback mechanism, thereby increasing the frequency of B10 progenitor cells. Moreover, patients with psoriasis showed levels of CD95 expression on all B cells and B10 progenitor cells that were comparable with those in healthy controls. CD95 expression is known to be upregulated by B-cell activation, and increased CD95 expression is associated with sensitivity to apoptosis [23]. This suggests that B10 progenitor cells are unlikely to be sensitive to apoptosis in patients with psoriasis. Furthermore, regardless the types of drugs used, therapeutic intervention resulting in significant improvement of cutaneous involvement in psoriasis appears to correct the abnormalities in B10 cells and B10 progenitor cells. This implies that patients with psoriasis may have some unknown factors in common that hinder the development of B10 cells. Further studies are necessary to clarify the precise mechanisms by which B10 cell development is obstructed in the pathogenesis of psoriasis.

Serum BAFF levels are reported to be elevated in autoimmune diseases, such as rheumatoid arthritis [24], systemic lupus erythematosus [25], and systemic sclerosis [26]. In the present study, serum BAFF levels were significantly higher in patients with psoriasis than in healthy controls, as reported previously [27]. Recent investigations have shown that BAFF enhances IL-10 secretion from murine $CD1d^{hi}CD5^{+}$ B10 progenitor cells in vitro [28]. Moreover, BAFF has also been shown to augment IL-10 production from human blood B cells [29], suggesting that BAFF may encourage B10 progenitor cells to produce IL-10. Interestingly, the administration of belimumab, a human mAb that neutralizes soluble BAFF, markedly reduced the transitional B-cell subset in

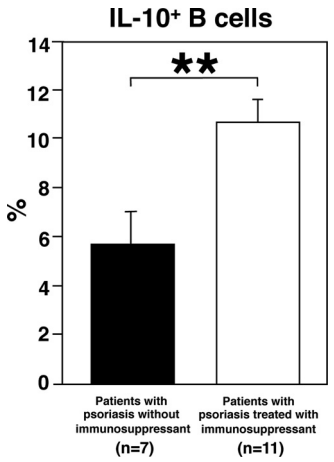

Fig. 6. The frequency of B10 cells in psoriasis patients after therapeutic intervention with immunosuppressants or only topical treatment. Samples of 11 psoriasis patients with therapeutic intervention with immunosuppressants and 7 psoriasis patients with topical therapy were analyzed. Bar graphs indicate mean (±SEM) percentages of B10 cells. Significant differences between sample means are indicated: $^{**}P < 0.01$.

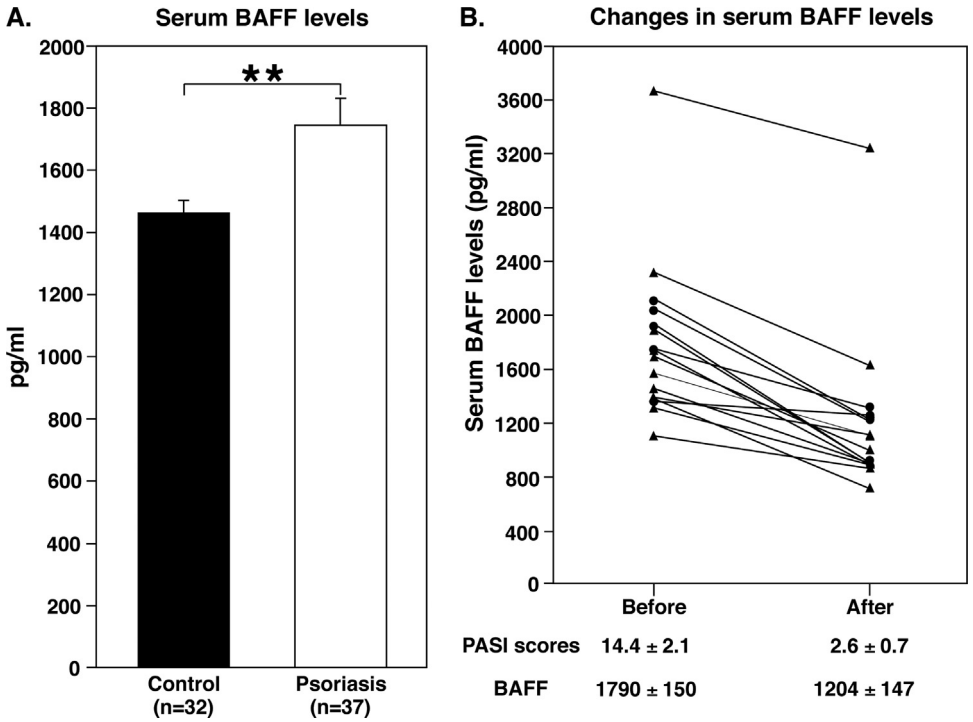

Fig. 7. Serum BAFF levels in patients with psoriasis and healthy controls (A). Changes in serum BAFF levels after initiation of therapeutic intervention with immunosuppressant (B). Samples of 37 patients with psoriasis and 32 healthy controls were examined. Bar graphs indicate mean (±SEM) serum BAFF levels (A). Significant differences between sample means are indicated: $^{**}P < 0.01$. Sixteen of the patients included in the serum BAFF levels study were followed up after treatments (B). Circles and triangles represent patients treated with ustekinumab and infliximab, respectively. $^{**}P < 0.01$ vs. serum BAFF levels at baseline. PASI scores and serum BAFF levels at baseline and after treatments are shown as mean (±SEM).

patients with systemic lupus erythematosus [30] and Sjögren syndrome [31], although these studies defined transitional B cells as $CD20^{+}CD10^{+}CD27^{-}$. Therefore, these results may also support the hypothesis that the pathogenesis of psoriasis involves some unknown abnormalities in the development of B10 cells from B10 progenitors, which increase serum BAFF levels to promote both B10 progenitor cell development and IL-10 production from B10 cells as a feedback mechanism. Further studies are required to determine the role of BAFF in the development of B10 cells in psoriasis.

Despite the decrease in B10 cells in patients with psoriasis, the serum IL-10 levels in these subjects were comparable with those in healthy controls. In mice, B10 cells are required to directly interact with T cells and selectively inhibit antigen-specific T-cell responses, without inducing systemic immunosuppression [32]. Therefore, IL-10 secretion from human B10 cells would be also restricted to the local site of inflammatory responses. Moreover, IL-10 is secreted by multiple cell types, including T cells, B cells, monocytes, macrophages, mast cells, and eosinophils [33], suggesting that IL-10 secretion from B10 cells might be too little to cause any change in the circulating IL-10 levels.

This study has some potential limitations. First, the number of subjects examined was small. Second, since age and sex might influence B cell homeostasis, unadjusted comparison of patients with psoriasis and healthy controls could yield biased results. Further studies with well-characterized larger populations are necessary to clarify the roles of B10 cells in the pathogenesis of psoriasis. Third, the phenotype of B10 cells in humans is still controversial and have not found the absolute markers of this population. As we showed in Fig. 2C, CD24 and CD38 were expressed at higher levels in IL-10^{+} than in IL-10^{-} B cells, while IL-10^{+} B cells also existed outside of the $CD24^{hi}CD38^{hi}$ gate, leading to the situation that the numbers of B10 cells occasionally exceeded B10 progenitors (Fig. 3). To circumvent this problem, it is essential to detect the B10 cell specific surface molecules or transcription factors such as FoxP3 in regulatory T cells and then re-evaluate the differences between patients with psoriasis and healthy individuals in the future study. Next, it would be worthwhile to further examine the functional abnormalities of B10 cells in vitro. Given their rarity in blood, B10 cells were extremely difficult to isolate in sufficient numbers for functional studies, with the current form of the protocol approved by the ethics committee. Nonetheless, the results of this study suggest the possible contributions of regulatory B cells in the pathogenesis of psoriasis. Furthermore, our results may provide new insights into B cell-based therapeutic approaches.

Conflict of interest

All authors have no conflicts of interest to declare.

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None.

References

[1] F.O. Nestle, D.H. Kaplan, J. Barker, Psoriasis, N. Engl. J. Med. 361 (2009) 496–509.
[2] M.J. Shlomchik, M.P. Madaio, D. Ni, M. Trounstine, D. Huszar, The role of B cells in lpr/lpr-induced autoimmunity, J. Exp. Med. 180 (1994) 1295–1306.
[3] J.D. Bouaziz, K. Yanaba, T.F. Tedder, Regulatory B cells as inhibitors of immune responses and inflammation, Immunol. Rev. 224 (2008) 201–214.
[4] K. Yanaba, J.-D. Bouaziz, K.M. Haas, J.C. Poe, M. Fujimoto, T.F. Tedder, A regulatory B cell subset with a unique $CD1d^{hi}CD5^{+}$ phenotype controls T cell-dependent inflammatory responses, Immunity 28 (2008) 639–650.

[5] T. Matsushita, K. Yanaba, J.D. Bouaziz, M. Fujimoto, T.F. Tedder, Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression, J. Clin. Invest. 118 (2008) 3420–3430.
[6] K. Yanaba, A. Yoshizaki, Y. Asano, T. Kadono, T.F. Tedder, S. Sato, IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model, Am. J. Pathol. 178 (2011) 735–743.
[7] K. Yanaba, J.D. Bouaziz, T. Matsushita, T. Tsubata, T.F. Tedder, The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals, J. Immunol. 182 (2009) 7459–7472.
[8] P.A. Blair, L.Y. Norena, F. Flores-Borja, D.J. Rawlings, D.A. Isenberg, M.R. Ehrenstein, et al., $CD19^{+}CD24^{hi}CD38^{hi}$ B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients, Immunity 32 (2010) 129–140.
[9] Y. Iwata, T. Matsushita, M. Horikawa, D.J. Dilillo, K. Yanaba, G.M. Venturi, et al., Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells, Blood 117 (2011) 530–541.
[10] J.D. Bouaziz, S. Calbo, M. Maho-Vaillant, A. Saussine, M. Bagot, A. Bensussan, et al., IL-10 produced by activated human B cells regulates $CD4^{+}$ T-cell activation in vitro, Eur. J. Immunol. 40 (2010) 2686–2691.
[11] F. Flores-Borja, A. Bosma, D. Ng, V. Reddy, M.R. Ehrenstein, D.A. Isenberg, et al., $CD19^{+}CD24^{hi}CD38^{hi}$ B cells maintain regulatory T cells while limiting T_H1 and T_H17 differentiation, Sci. Transl. Med. 5 (2013) 173ra23.
[12] C.I. Daien, S. Gailhac, T. Mura, R. Audo, B. Combe, M. Hahne, et al., Regulatory B10 cells are decreased in patients with rheumatoid arthritis and are inversely correlated with disease activity, Arthritis Rheumatol. 66 (2014) 2037–2046.
[13] N. Gao, J. Dresel, V. Eckstein, R. Gellert, H. Storch, R.K. Venigalla, et al., Impaired suppressive capacity of activation-induced regulatory B cells in systemic lupus erythematosus, Arthritis Rheumatol. 66 (2014) 2849–2861.
[14] H.Q. Zhu, R.C. Xu, Y.Y. Chen, H.J. Yuan, H. Cao, X.Q. Zhao, et al., Impaired function of $CD19^{+}$ $CD24^{hi}$ $CD38^{hi}$ regulatory B cells in patients with pemphigus, Br. J. Dermatol. 172 (2015) 101–110.
[15] A. Saussine, A. Tazi, S. Feuillet, M. Rybojad, C. Juillard, A. Bergeron, et al., Active chronic sarcoidosis is characterized by increased transitional blood B cells, increased IL-10-producing regulatory B cells and high BAFF levels, PLoS One 7 (2012) e43588.
[16] S.K. Todd, R.J. Pepper, J. Draibe, A. Tanna, C.D. Pusey, C. Mauri, et al., Regulatory B cells are numerically but not functionally deficient in anti-neutrophil cytoplasm antibody-associated vasculitis, Rheumatology (Oxford) 53 (2014) 1693–1703.
[17] K. Yanaba, M. Kamata, N. Ishiura, S. Shibata, Y. Asano, Y. Tada, et al., Regulatory B cells suppress imiquimod-induced, psoriasis-like skin inflammation, J. Leukoc. Biol. 94 (2013) 563–573.
[18] K. Asadullah, W. Sterry, K. Stephanek, D. Jasulaitis, M. Leupold, H. Audring, et al., IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach, J. Clin. Invest. 101 (1998) 783–794.
[19] E. Weiss, A.J. Mamelak, S. La Morgia, B. Wang, C. Feliciani, A. Tulli, et al., The role of interleukin 10 in the pathogenesis and potential treatment of skin diseases, J. Am. Acad. Dermatol. 50 (2004) 657–675 quiz 76-8.
[20] S. Dass, E.M. Vital, P. Emery, Development of psoriasis after B cell depletion with rituximab, Arthritis Rheum. 56 (2007) 2715–2718.
[21] F. Mielke, J. Schneider-Obermeyer, T. Dorner, Onset of psoriasis with psoriatic arthropathy during rituximab treatment of non-Hodgkin lymphoma, Ann. Rheum. Dis. 67 (2008) 1056–1057.
[22] S.L. Kalled, Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production, Semin. Immunol. 18 (2006) 290–296.
[23] J. Wang, T. Watanabe, Expression and function of Fas during differentiation and activation of B cells, Int. Rev. Immunol. 18 (1999) 367–379.
[24] G.S. Cheema, V. Roschke, D.M. Hilbert, W. Stohl, Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases, Arthritis Rheum. 44 (2001) 1313–1319.
[25] J. Zhang, V. Roschke, K.P. Baker, Z. Wang, G.S. Alarcon, B.J. Fessler, et al., Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus, J. Immunol. 166 (2001) 6–10.
[26] T. Matsushita, M. Hasegawa, K. Yanaba, M. Kodera, K. Takehara, S. Sato, Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes, Arthritis Rheum. 54 (2006) 192–201.
[27] S. Samoud-El Kissi, Y. Galai, R. Sghiri, N. Kenani, N. Ben Alaya-Bouafif, J. Boukadida, et al., BAFF is elevated in serum of patients with psoriasis: association with disease activity, Br. J. Dermatol. 159 (2008) 765–768.
[28] M. Yang, L. Sun, S. Wang, K.H. Ko, H. Xu, B.J. Zheng, et al., Novel function of B cell-activating factor in the induction of IL-10-producing regulatory B cells, J. Immunol. 184 (2010) 3321–3325.
[29] D. Saulep-Easton, F.B. Vincent, P.S. Quah, A. Wei, S.B. Ting, C.M. Croce, et al., The BAFF receptor TACI controls IL-10 production by regulatory B cells and CLL B cells, Leukemia 30 (2016) 163–172.
[30] A.M. Jacobi, W. Huang, T. Wang, W. Freimuth, I. Sanz, R. Furie, et al., Effect of long-term belimumab treatment on B cells in systemic lupus erythematosus: extension of a phase II, double-blind, placebo-controlled, dose-ranging study, Arthritis Rheum. 62 (2010) 201–210.
[31] E. Pontarini, M. Fabris, L. Quartuccio, M. Cappeletti, F. Calcaterra, A. Roberto, et al., Treatment with belimumab restores B cell subsets and their expression of B cell activating factor receptor in patients with primary Sjogren's syndrome, Rheumatology (Oxford) 54 (2015) 1429–1434.
[32] A. Yoshizaki, T. Miyagaki, D.J. DiLillo, T. Matsushita, M. Horikawa, E.I. Kountikov, et al., Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions, Nature 491 (2012) 264–268.
[33] K. Asadullah, W. Sterry, H.D. Volk, Interleukin-10 therapy—review of a new approach, Pharmacol. Rev. 55 (2003) 241–269.