Serum level of APRIL/BLyS in Behçet’s disease patients: clinical significance in uveitis and disease activity

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Abstract

Objective The aim of the study reported here was to assess the serum concentration of B-cell activating factors, B lymphocyte stimulators (BLyS), and a proliferation-inducing ligand (APRIL) in Behçet disease (BD) patients in order to evaluate their role and study their relation to uveitis subtype and disease activity.

Methods The study included 58 consecutively recruited BD patients with a mean age of 35.54 ± 8.85 years and disease duration of 8.33 ± 5.84 years and 30 age- and sex-matched controls. Disease activity was assessed using the BD current activity form score. Patients were subclassified according to the presence and type of uveitis. Serum BLyS and APRIL were measured by enzyme-linked immunosorbent assay.

Results Recurrent uveitis was present in 42 (72.41 %) patients, of whom 19 had the anterior form, 13 had the posterior form, and ten had a combined anterior with posterior form; 16 had no eye involvement. Serum APRIL (60.29 ± 57.99 ng/ml) and BLyS (2.31 ± 1.97 ng/ml) levels were significantly higher in the BD patients than in the controls (4.14 ± 4.31 and 0.49 ± 0.39 ng/ml, respectively; *P* < 0.0001). The levels were clearly higher in those with combined uveitis. The BLyS level significantly correlated with disease activity.

Conclusions The overexpression of BLyS and APRIL in BD patients supports the notion of a critical role for B cell activation factors in BD, particularly in terms of uveitis and disease activity. This provides an interesting prospect for the use of anti-BLyS/APRIL antibodies against future therapeutic targets.

Keywords Behçet’s disease · Uveitis · APRIL · BLyS

Introduction

The treatment of Behçet’s disease (BD) is frequently associated with challenges, particularly in cases with eye involvement. Recurrent oral and genital aphthous ulcerations are the hallmarks of BD. Other organs can be involved, but ocular disease is one of its main morbidities [1] and considered to be the most difficult to manage [2], although some difficulties in the treatment of retinal vasculitis still remain [1].

It is highly likely that the importance of immunological data in the context of BD treatment will increase as immunological mechanisms are currently attracting the most research interest [3]. Cell-mediated immunity, rather than immune complex deposition, is considered to be responsible for the perpetuation of BD ocular inflammation, with CD4+ T lymphocytes playing a central role in this process. It has also been reported that B lymphocytes are present only infrequently in BD [4], and there have been descriptions of abnormalities in lymphocyte subpopulations [5]. Plasma cell infiltration is observed in recurrent arthritis associated with BD, but the immune mechanism underlying synovial B lymphocyte proliferation remains unclear [6]. Increased immunoglobulin (Ig)-secreting B
cells and elevated Ig levels have been described, and a modified B cell function in BD has been suggested [7].

Our understanding of the mechanisms underlying B cell proliferation and survival have been enhanced since the identification of two tumor necrosis factor (TNF) superfamily members, namely, the B-cell activating factor belonging to the TNF family (BAFF) and the B cell-activating factor, also known as B lymphocyte stimulator (BlyS), and a proliferation-inducing ligand (APRIL) [8]. Both molecules are key survival factors during B cell maturation and essential for the development of B cell tolerance. They regulate the size and composition of the B cell compartment and act as important driving factors for B cell hyperplasia and autoantibody production in autoimmune processes [9]. Serum levels of the BlyS are elevated in patients with systemic autoimmune diseases [10]. In a previous study, increased BlyS and APRIL serum levels in juvenile idiopathic arthritis patients suggested their possible role in this disease [11]. A role of B cells, as well as other infiltrating cells, in the pathogenesis of BD is indicated based on the histopathology of central nervous system (CNS) lesions [12]. BlyS has been found to be expressed in the CNS of patients with neuro-BD [13]. It has also been found that BlyS is produced in BD patients having a pulmonary manifestation [14]. Furthermore, BlyS and its signaling in B cells have been found to contribute to B cell abnormalities and the development of skin disease in BD patients [10].

Earlier studies suggested that B cell abnormalities, some of which are associated with disease activity, may be involved in the pathogenesis of BD [15]. Rituximab is a chimeric monoclonal antibody that acts against the specific B cell antigen CD20. The recent success of rituximab in the treatment of autoimmune diseases, which are mostly considered to be T cell mediated, indicates that B cells must play a much broader role in the pathogenesis of autoimmune diseases than has been generally appreciated to date [1].

As (1) uveitis is a leading manifestation of BD, (2) B cells are being given a larger role in disease pathogenesis, and (3) a role for APRIL and BlyS in autoantibody production has been suggested, the aim of our study was to study the serum concentration of APRIL and BlyS in relation to the type of uveitis and correlate them to disease activity.

Methods

Patients

Fifty-eight consecutively recruited BD patients satisfying the International Study Group for Behçet’s Disease new set of diagnostic criteria [16] were enrolled in our study. These patients were recruited consecutively from the Rheumatology and Ophthalmology departments and outpatient clinics, Cairo University hospitals. They had a mean age of 35.54 ± 8.85 years and mean disease duration of 8.33 ± 5.84 years. Thirty age- and sex-matched healthy volunteers served as controls. A full medical history was obtained from all patients, and all patients were given a clinical examination. The appropriate laboratory tests were also carried out. Medications received by the patients were noted, and patients receiving corticosteroids for management of their disease were not excluded from the study. Disease activity was assessed using the Behçet disease current activity form (BDCAF) [17] score. The patients were classified according to type of uveitis, i.e., those with the anterior or posterior form, those with both forms, and those without uveitis at the time of sampling.

Serum BlyS and APRIL determination

All sera were preabsorbed with protein A (Amersham Biosciences, Piscataway, NJ) to deplete Ig. BlyS and APRIL serum levels were analyzed by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the respective manufacturer’s protocol (APRIL: Bender Medsystems, Vienna, Austria; BlyS: Quantikine; R&D System, Minneapolis, MN). The detection limit of the assay was determined to be 0.4 ng/ml, and the intra-assay and inter-assay coefficients of variation in the APRIL ELISA kit were 8.1 and 7.1 %, respectively. For the BlyS assay, the mean coefficient of variation was 8.4 % and the lower limit of detection was 0.78 ng/ml. The patients’ written consent was obtained according to the declaration of Helsinki, and the study was approved by the local ethics committee prior to their inclusion in the study.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) ver. 10 (LEAD Technology, Charlotte, NC) was used to analyze the data. Continuous variables were summarized by their mean ± standard deviation [median (minimum–maximum)], and categorical variables were summarized using absolute values and percentages. The nonparametric Mann–Whitney U test was used to compare two independent groups and the Kruskal–Wallis test was used to compare more than two groups. Spearman’s rank correlation test was used as a measure of association of quantitative variables. Two-tailed P values of ≤0.05 were considered to be statistically significant.

Results

The study included 58 BD patients, of whom 48 were men and ten were women. Uveitis was present in 42 (72.41 %)
patients, while 16 had no eye involvement at the time of sampling. Anterior uveitis was present in 19 patients, posterior uveitis in 13 patients, and combined anterior with posterior uveitis in ten patients. In addition to uveitis, other eye manifestations included corneal ulcers (6 patients), hypopyon (3 patients), macular edema (6 patients), and retinal vasculitis (2 patients). There was diminution of vision in nine patients with posterior uveitis, three of which had vitreous hemorrhage and two had glaucoma. Thirty age- (mean age 32.87 ± 5.05 years and sex- (25 men, 5 women) matched healthy volunteers were included as controls. The demographic and clinical features of the BD patients are presented in Table 1. Of the 58 BD patients, 53 received steroids, 51 received colchicines, four received azathioprine, two received methotrexate, three received cyclosporine, two received cyclophosphamide, and five received chlorambucil. Two patients were receiving biological agents: infliximab (3 mg/kg intravenous infusion at 0, 2, and 6 weeks and then every 8 weeks) and adalimumab (40 mg subcutaneous every 2 weeks).

The clinical response and changes in the level of the APRIL and BLyS in the two patients receiving a biological agent were monitored 6 and 14 weeks post-administration. In the patient receiving infliximab, the initial serum levels of APRIL and BLyS were measured at 2 weeks post-drug administration and followed up at the 6th and 14th week post-administration. The serum level of APRIL did not show an obvious decrease, being 117.8 ng/ml initially and decreasing to 109.2 ng/ml after 6 weeks and 99.6 ng/ml after 14 weeks. The serum level of BLyS notably decreased with infliximab treatment, being initially 2.31 and then decreasing to 1.87 ng/ml at 6 weeks post-drug administration and to 1.32 ng/ml after 14 weeks. The patient receiving infliximab showed a lag in clinical improvement, starting at the 10th week post-infliximab administration, and the BDCAF showed a mild tendency to decrease (from 5 to 4). In the other patient a biological agent (adalimumab), the serum APRIL level obviously decreased from 158.1 ng/ml to reach 70.6 and 29.3 ng/ml at 6 and 14 weeks post-drug administration, respectively. Similarly, the serum BLyS level decreased from 3.98 ng/ml to reach 1.61 and 0.87 ng/ml after 6 and 14 weeks of treatment, respectively. This improvement in the measured laboratory parameters was associated with a remarkable clinical improvement that was marked by disease remission at the 14th week. The BDCAF decreased from an initial score of 5 to reach 0 after 14 weeks. The other medications did not affect APRIL/BLyS levels.

The mean serum APRIL level was significantly higher in the BD patients (60.29 ± 57.99 ng/ml) [31.24 (2.21–245)] than in the controls (4.14 ± 4.31 ng/ml) [3.02 (0.99–17.6)] (P < 0.0001). The mean serum BLyS level was also significantly higher in BD patients (2.31 ± 1.97 ng/ml) [1.61 (0.1–6.61)] than in the controls (0.49 ± 0.39 ng/ml) [0.42 (0–1.3)] (P < 0.0001), as presented in Fig. 1. The disease activity score (BDCAF) was significantly higher in those BD patients with posterior uveitis (3.92 ± 0.95) compared to those with anterior (1.79 ± 1.4) or combined uveitis (2.7 ± 1.49) (P < 0.001). The serum APRIL level was significantly higher in those BD patients with combined anterior/posterior uveitis (118.51 ± 64.03 ng/ml) than in those with either anterior (49.03 ± 55.23 ng/ml) or posterior (55.62 ± 51.58 ng/ml) uveitis (P 0.003). Similarly, the serum BLyS level was significantly higher in those with combined uveitis (3.72 ± 1.75 ng/ml) than in those with anterior (2.34 ± 2.06 ng/ml) or posterior (2.6 ± 1.83 ng/ml) (0.007). Figure 1 shows the different levels of APRIL and BLyS in patients and controls. The serum APRIL level was significantly higher in those with corneal ulcers (132 ± 68 ng/ml) than in those without (52.01 ± 51.29 ng/ml) (P 0.03), while the serum BLyS level was highest in the two patients with retinal vasculitis.

The serum albumin level was significantly lower and the daily steroid dose was higher in BD patients with combined uveitis than in those without [4.04 ± 0.28 vs. 4.27 ± 0.24 g/dl (P 0.046) and 26.5 ± 10.55 vs. 12.66 ± 8.34 (P < 0.0001)]. There was no significant difference in the other studied parameters. There was no significant different between the studied parameters according to gender.

There were no correlations of significance between the APRIL levels with the studied disease parameters. However, there was a tendency to a correlation between serum APRIL level and steroid dose (r = 0.241, P < 0.069), while the BLyS level significantly correlated with the BD activity

<table>
<thead>
<tr>
<th>Feature in BD patients (n = 58)</th>
<th>Mean ± SD [median (min–max)]</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>35.54 ± 8.85 [37 (21–54)]</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>8.33 ± 5.84 [6 (1.5–20)]</td>
</tr>
<tr>
<td>BDCAF</td>
<td>2.59 ± 1.52 [2 (1–5)]</td>
</tr>
<tr>
<td>Steroids [n and dose (mg/day)]</td>
<td>53/58 [15 (2.5–40)]</td>
</tr>
<tr>
<td>Colchicine [n and dose (mg/day)]</td>
<td>51/58 [1 (0.5–1.5)]</td>
</tr>
<tr>
<td>Eye manifestations</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Uveitis</td>
<td>42 (72.41)</td>
</tr>
<tr>
<td>Anterior</td>
<td>19 (32.76)</td>
</tr>
<tr>
<td>Posterior</td>
<td>13 (22.41)</td>
</tr>
<tr>
<td>Anterior/posterior</td>
<td>10 (17.24)</td>
</tr>
<tr>
<td>Corneal ulcers</td>
<td>6 (10.34)</td>
</tr>
<tr>
<td>Hypopyon</td>
<td>3 (5.17)</td>
</tr>
<tr>
<td>Retinal vasculitis</td>
<td>2 (3.45)</td>
</tr>
</tbody>
</table>

BDCAF Behçet disease current activity form, SD standard deviation
addition, the BLyS level significantly correlated with the BD activity. The serum APRIL level was higher in those BD patients with corneal ulcers, while the two patients with retinal vasculitis had a higher BLyS level. Similarly, in active BD patients with pulmonary manifestations, the increased antigen-induced production of BLyS may contribute to Ig synthesis by B cells. Moreover, the cells existing in the lung might affect each other through BLyS [14]. In an earlier study, serum BLyS levels were higher in active BD patients and correlated with the extent of skin lesions, while disease remission was associated with a reduction in serum BLyS level. BLyS receptor expression on B cells has been found to be higher in BD patients with vasculitis [10]. In a study involving BD patients receiving rituximab, there was a significant decrease in disease activity and a remarkable efficiency, with improvement of such severe ocular manifestations as posterior uveitis and retinal vasculitis, compared to those receiving cytotoxic combination therapies [2]. In a case report, one patient with visual loss due to retinal vasculitis who was resistant to prednisolone and azathioprine was treated successfully with rituximab, and remission was sustained for 2 years [1]. In our study, the serum BLyS level significantly correlated with the white blood cell count, while in another study, the BLyS levels considerably correlated with lymphocyte, neutrophil, and macrophage counts [14].

Of our BD patients with uveitis, six had corneal ulcers. In a related study, histopathological study of corneal ulcers in BD patients revealed infiltrating T-lymphocytes admixed with B cells [18]. In a previous study on different types of uveitis, including those of BD, both T and B
lymphocytes were identified in the aqueous humor [19]. In our study, there was only a tendency for a significant correlation between the serum APRIL level and steroid dose in BD patients. This is in accordance with the finding that the number of B cells in BD patients is not influenced by treatment with steroids or immunosuppressives [19].

The significant correlation of BLyS with disease activity in BD patients throws light on the importance of targeting this molecule in the management of this disease. In another study, the elevated level of BLyS in scleroderma patients confirmed the importance of developing new therapeutic targets for its inhibition as a means to slow disease progression. Similarly, the well-known role of BLyS in the pathogenesis and disease activity of systemic lupus erythematosus and the novel noticeable correlation between BLyS level and the damage index have highlighted the importance of utilizing BLyS as an indicator of disease damage and a predictor of poor outcome [20]. In a recent study on the serum levels of APRIL/BLyS in patients with an IgG4-related disease (IgG4RD), APRIL/BLyS levels were found to be useful markers for predicting disease activity, suggesting the need for further studies to elucidate their role in disease pathogenesis [21].

In conclusion, our results support the notion that B cell activation factors play a critical role in BD and provide new information on the pathogenesis of BD uveitis. Our findings further place B cells in a pivotal role and call for additional research to elucidate the intrinsic mechanisms explaining APRIL and BLyS overexpression and their relation to disease activity. They also create a hope that anti-BLyS/APRIL antibodies can be used against future therapeutic targets. Research on a larger scale and longitudinal studies are recommended to confirm the present results. Future studies should assess BLyS levels in other ocular diseases in comparison to those in BD.

Conflict of interest None.

References