Interferon-α-induced B-lymphocyte stimulator expression and mobilization in healthy and systemic lupus erythematosus monocytes

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Abstract

Objective. The aim of this study was to investigate the cellular populations and regulatory factors responsible for B-lymphocyte stimulator (BLyS) overexpression in SLE patients.

Methods. Surface and intracellular BLyS levels were quantified by flow cytometry in healthy and SLE monocytes cultured in the presence of TNF-α, IFN-α, IFN-γ, GM-CSF and SLE immune complexes (SLE-ICs), while soluble BLyS was measured by ELISA. Also, both surface and intracellular BLyS expression by different cell subsets was determined in 23 SLE patients and 16 healthy controls. Disease activity was assessed using classic BILAG index.

Results. In vitro experiments using healthy monocytes showed that IFN-α and SLE-ICs induced a progressive increase in surface-bound BLyS with respect to the intracellular stores. IFN-α-treated SLE monocytes, especially from patients with high anti-dsDNA levels or disease activity, exhibited higher intracellular levels of BLyS that was mobilized to the membrane more rapidly and subsequently released. Furthermore, ex vivo analysis of SLE patients revealed up-regulated BLyS expression in B cells, myeloid and plasmacytoid dendritic cells (DCs), whereas active patients had an increased surface:intracellular BLyS ratio in monocytes and myeloid DCs.

Conclusion. Monocyte BLyS induction and mobilization from intra- to extracellular compartments seems to be influenced by IFN-α and disease activity or anti-dsDNA levels. Accordingly, monocytes and myeloid DCs from active patients presented the highest membrane-bound:intracellular BLyS ratio. In addition, expression levels in several blood cells support the existence of generalized immune stimulation in SLE patients.

Key words: systemic lupus erythematosus, BLyS, BAFF, IFN-α, myeloid cells.

Introduction

SLE is an autoimmune disease with heterogeneous clinical manifestations characterized by systemic activation involving B and T lymphocytes and myeloid cells [1, 2], as well as the presence of pathogenic autoantibodies against DNA and other nuclear antigens [3, 4]. In spite of the unknown causes of SLE, evidence suggests that alterations in the production of cytokines play an important role in the pathogenesis of the disease, contributing to immune dysfunction, inflammation and organ damage [5]. Of these cytokines, IFN-α plays a central role in SLE [6-9], since a large subgroup of patients present with increased IFN-α serum levels or IFN-α-induced genes, usually correlated with disease activity and the generation of autoantibodies [10-13]. In the same way, TNF-α is a cytokine well known for its role in the regulation of inflammation and apoptosis, two processes involved in the pathogenesis of SLE [14, 15]. Several studies have shown higher TNF-α serum levels in SLE patients,
frequently linked to disease activity [16–18] or to specific immunological or clinical features such as elevated autoantibody production [19] and LN [20–22]. Additionally IL-1β, GM-CSF and IFN-γ, among others, are also dysregulated in SLE [23–25].

In recent years, several studies have demonstrated the critical contribution of B-lymphocyte stimulator (BLYS), also known as B cell activating factor, to SLE pathogenesis. BLYS is a member of the TNF family of ligands, playing an essential role in the survival, differentiation and antibody production of B cells [26]. Transgenic mice overexpressing this cytokine develop autoimmunity with lupus-like symptoms associated with elevated circulating titres of anti-dsDNA autoantibodies and accelerated GN [27–29]. In addition, mice with spontaneous SLE-like diseases, such as the NZBxNZW and MRL-lpr/lpr models, have increased levels of BLYS in their serum [28]. Also, clinical studies have confirmed the presence of increased serum levels of BLYS in SLE patients correlating with the activity of the disease [30–34]. Furthermore, therapy with a BLYS-neutralizing monoclonal antibody has shown efficacy in reducing disease activity and has been approved for the treatment of SLE patients [35].

It has been documented that BLYS mRNA expression in SLE blood leucocytes correlated better with disease severity than the BLYS concentration in blood [36, 37], suggesting that blood leucocytes may be a major source of BLYS in SLE. In this regard, this cytokine is known to be expressed by a wide variety of cells of myeloid origin, including monocytes, neutrophils, plasmacytoid dendritic cells (pDCs) and myeloid DCs (mDCs), but its expression is dependent on the activation state, usually being low or null on resting cells [37–39]. Although BLYS is produced as a membrane-bound form as well as a soluble protein [40, 41], previous findings have confirmed that BLYS is also stored intracellularly in human neutrophils and that several cytokines and pro-inflammatory mediators can release soluble BLYS in vitro and in vivo [42, 43]. However, it remains unclear which stimuli and cellular populations are responsible for BLYS overexpression in human SLE.

Hence the aim of the present study was to analyse in vitro the regulation of BLYS expression and release in healthy and SLE mononuclear cells under classic pro-inflammatory lupus mediators, such as cytokines and SLE immune complexes (SLE-ICs). In addition, we evaluated the contribution of different subsets of fresh cells (pDCs, mDCs, B cells, neutrophils and monocytes) to the levels of BLYS in SLE patients. These data will allow us to understand the effect of the main aetiopathological SLE factors on BLYS production and release.

Methods

Patients and healthy controls
All patients included in the study fulfilled at least four ACR revised criteria for the classification of SLE [44]. Sixteen patients [15 female, 1 male; mean age 46.03 years (s.d. 12.33)] from the Internal Medicine Autoimmune Disease Unit (Hospital Universitario Central de Asturias) and nine healthy individuals were enlisted in the in vitro experiments performed in this study. Disease duration was 13.80 years (s.d. 10.65). Eight patients were taking steroids, 14 patients were taking chloroquine and 2 were treated with other immunosuppressive medication. For the ex vivo BLYS analysis, 23 patients from the Birmingham SLE cohort and 16 sex- and age-matched healthy blood donors were recruited. Information on clinical features during the disease course was obtained after a detailed review of clinical histories (Table 1). Anti-dsDNA titre and disease activity (BILAG) [45] were determined at the time of sampling in all SLE patients. A BILAG system score of ≥B (≥5 points) was considered a marker of active disease [46]. Ethics approval for this study was obtained from the Regional Ethics Committee for Clinical Research (Servicio de Salud del Principado de Asturias) as well as from the South Birmingham and Black Country Research Ethics Committees according to the Declaration of Helsinki. Written informed consent was obtained from healthy blood donors and SLE patients prior to participation in the study.

Isolation of immune complexes
SLE-ICs were obtained by polyethylene glycol (PEG) precipitation from SLE patient serum. Sera from SLE patients was treated with an equal volume of 5% PEG 6000 with 0.1 mol/l EDTA (Sigma Chemical, St Louis, MO, USA) and left to stand at 4°C overnight. After that, SLE-ICs were centrifuged for 1 h at 1100 g and washed twice in sterile PBS, then diluted to the initial serum volume in sterile PBS.

<table>
<thead>
<tr>
<th>Variable</th>
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<tr>
<td>Total SLE patients, n</td>
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</tr>
<tr>
<td>Sex, female/male</td>
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</tr>
<tr>
<td>Age, mean (s.d.), years</td>
<td>40.13 (10.92)</td>
</tr>
<tr>
<td>Disease duration, mean (s.d.), years</td>
<td>9.82 (6.67)</td>
</tr>
<tr>
<td>ACR criteria at diagnosis, mean (s.d.)</td>
<td>5.43 (1.38)</td>
</tr>
<tr>
<td>Anti-dsDNA titre, mean (s.d.), U/ml</td>
<td>60.16 (156.68)</td>
</tr>
<tr>
<td>BILAG index, mean (s.d.)</td>
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</tr>
<tr>
<td>Ethnic background, n</td>
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</tr>
<tr>
<td>Asian</td>
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</tr>
<tr>
<td>Afro-Caribbean</td>
<td>5</td>
</tr>
<tr>
<td>Oriental</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>Treatment, n</td>
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<tr>
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</tr>
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<tr>
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<tr>
<td>Immunosuppressive drugs</td>
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<td>MTX</td>
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<td>MMF</td>
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Table 1 Characteristics and disease parameters from SLE patients

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Purification and in vitro culture of monocytes

Human peripheral blood mononuclear cells (PBMCs) from healthy donors and patients were obtained by centrifugation over Ficoll-Hypaque gradients (Lymphoprep, Nycomed, Oslo, Norway). Monocytes were isolated from previously obtained PBMCs by negative selection using the Human Monocyte Enrichment Kit, according to the protocol provided by EasySep (StemCell Technologies, Vancouver, BC, Canada).

Monocytes, at a density of $2 \times 10^6$/ml were treated with a range of cytokines: GM-CSF (100 U/ml), TNF-α (10 ng/ml), IL-1β (50 ng/ml), IFN-γ (100 U/ml) (all provided by R&D Systems, Abingdon, UK), human IFN-α2b (Intron-A, 1000 U/ml) and purified SLE-ICs (50% of the total volume of culture). These cytokine concentrations did not exhibit cytotoxic effects as were previously tested in our laboratory. The cells were cultured in complete RPMI medium (RPMI 1640 containing 2 mM L-glutamine and 25 mM HEPES, supplemented with 10% heat-inactivated fetal calf serum and the antibiotics streptomycin and ampicillin at 100 μg/ml) at 37°C and 5% carbon dioxide. At different times of culture (0.5, 2, 4, 6 and 12 h), 100 μl of cell-free supernatants from these cultures were collected for BLyS quantification and the corresponding monocyte pellets were harvested for intra- and extracellular cytometric analysis.

Monoclonal antibodies

Mouse monoclonal antibodies specific for CD303 (BDCA-2) (PE) (Miltenyi Biotec, Bergisch Gladbach, Germany), CD14 (PE), CD19 (PE-Cy7), BLyS (FITC), CD123 (APC), CD1c (BDCA-1) (APC) and isotype, concentration and fluorochrome-matched control antibodies were purchased from eBioscience (San Diego, CA, USA).

Flow cytometric analysis of BLyS

In vitro cultured monocytes were collected from culture plates and stained for CD14 expression. Peripheral blood samples from controls and patients were collected with EDTA as anticoagulant, 100 μl aliquots of venous blood were diluted with cold PBS with 2% BSA and cells were spun down to remove serum twice. To identify pDCs, cells were stained with anti-BDCA-2 and anti-CD123. mDCs were identified as CD19- BDCA-1+, while B cells and monocytes were identified by the expression of CD19 or CD14, respectively. Neutrophils were identified according to their distinctive forward- and side-scatter signal.

For analysis of intracellular BLyS expression, both fresh and in vitro cultured cells were fixed and permeabilized after staining for extracellular markers and incubated with anti-BLys (FITC)-labelled monoclonal antibodies (Fix & Perm Kit, Caltag Laboratories, Carlsbad, CA, USA). All washing steps were performed in PBS containing 2% BSA. All experiments were controlled using species-, isotype- and concentration-matched irrelevant antibodies. Analyses were carried out by acquisition of 200,000 events/tube on a Coulter CyAn Flow Cytometer (Beckman Coulter, Fullerton, CA, USA). Samples were subsequently analysed using FlowJo software (Scripps Research Institute, San Diego, CA, USA). Results were expressed as the median fluorescence intensity of gated populations after subtracting the fluorescence median of the respective isotype control (ΔMFI).

BLyS and IFN-α protein quantification

Culture supernatants and serum samples were collected and maintained at −80°C until cytokine determination was carried out. Expression of BLyS by cultured monocytes was determined using a commercial ELISA (eBioscience) according to the manufacturer’s instructions. IFN-α serum levels were quantified by flow cytometry using BD Cytometric Bead Array (CBA) Flex Set (Becton Dickinson, San Jose, CA, USA) following the manufacturer’s instructions. The lower limit of detection was 0.13 ng/ml for BLyS and 1.5 pg/ml for IFN-α.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normal distribution of the data. Data from in vitro experiments were represented by mean (S.E.M.) and differences between culture conditions were assessed by the paired t-test or analysis of variance (ANOVA) and Tukey’s post hoc test, as described in the figure legends; correlation was examined by Pearson’s rank correlation test. ΔMFI of BLyS expression by fresh cells is expressed as the median value (interquartile range) and non-parametric testing was used to determine differences between patient groups (Mann–Whitney U-test), while correlation was examined by Pearson’s rank correlation test. Data were analysed using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA) and SPSS 18.0 statistical software package (SPSS, Chicago, IL, USA) and P-values < 0.05 were considered significant.

Results

IFN-α and immune complexes induce BLyS expression and mobilization in healthy monocytes

First, we wanted to test in vitro the influence of some relevant pathogenic factors in SLE on the regulation of BLyS expression and subsequent release. To this end, monocytes isolated from the peripheral blood of healthy donors were cultured with a range of cytokines (TNF-α, IFN-α, IFN-γ, GM-CSF) as well as immune complexes freshly isolated from SLE sera (SLE-ICs). Cells were harvested at different times and intracellular and surface-bound BLyS expression were determined separately by flow cytometry, whereas soluble BLyS was quantified in the supernatants by ELISA. The results of flow cytometry (Table 2) showed that of all tested stimuli, IFN-α and SLE-IC treatments were the most efficient inducers of BLyS production analysing both cellular compartments. In particular, we observed that intracellular BLyS levels after IFN-α or SLE-IC treatment peaked at 2 h, followed by a significant increase in
membrane-bound BLyS expression at latter times. These data suggest that IFN-α and SLE-ICs induced early up-regulation of the intracellular levels of BLyS in healthy monocytes, which was subsequently mobilized to the extracellular compartment, leading to the alteration in the extra- to intracellular balance (Fig. 1A). In fact, the extracellular:intracellular BLyS ratio after 6 h of culture with IFN-α was significantly higher than in unstimulated monocytes. In accordance with these results, quantification of the soluble BLyS released to the culture supernatant showed the highest levels after 6 h of IFN-α and SLE-IC treatment (Fig. 1B).

### Table 2 BLyS expression in stimulated healthy monocytes

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>TNF-α</th>
<th>IFN-α</th>
<th>IFN-γ</th>
<th>GM-CSF</th>
<th>SLE-ICs</th>
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<tr>
<td><strong>Intracellular</strong></td>
<td></td>
<td></td>
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<tr>
<td>Basal: 26.97 (3.90)</td>
<td></td>
<td></td>
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<tr>
<td>0.5 h</td>
<td>22.06 (3.90)</td>
<td>26.09 (1.81)</td>
<td>26.84 (2.28)</td>
<td>27.85 (2.97)</td>
<td>26.08 (3.41)</td>
<td>25.90 (3.33)</td>
</tr>
<tr>
<td>2 h</td>
<td>37.66 (2.96)</td>
<td>34.51 (4.47)</td>
<td>47.53 (4.41)**</td>
<td>36.50 (4.90)</td>
<td>39.20 (4.76)</td>
<td>46.00 (8.13)*</td>
</tr>
<tr>
<td>4 h</td>
<td>30.97 (2.37)</td>
<td>29.25 (4.27)</td>
<td>38.49 (3.10)</td>
<td>22.08 (3.33)</td>
<td>22.60 (4.08)</td>
<td>32.60 (2.74)</td>
</tr>
<tr>
<td>6 h</td>
<td>25.91 (2.23)</td>
<td>35.75 (4.52)</td>
<td>27.24 (2.51)</td>
<td>26.39 (0.90)</td>
<td>29.51 (7.16)</td>
<td>20.57 (4.15)</td>
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<tr>
<td>12 h</td>
<td>31.23 (2.52)</td>
<td>34.62 (4.06)</td>
<td>35.53 (4.40)</td>
<td>35.47 (3.50)</td>
<td>34.19 (2.99)</td>
<td>33.31 (8.52)</td>
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<tr>
<td><strong>ANOVA P-value</strong></td>
<td>0.1730</td>
<td>0.6503</td>
<td>0.0013</td>
<td>0.3320</td>
<td>0.2056</td>
<td>0.0821</td>
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<td><strong>Extracellular</strong></td>
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<tr>
<td>Basal: 17.67 (2.00)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.5 h</td>
<td>18.79 (1.81)</td>
<td>19.71 (1.19)</td>
<td>19.34 (1.70)</td>
<td>20.69 (2.06)</td>
<td>19.99 (2.77)</td>
<td>13.08 (0.90)</td>
</tr>
<tr>
<td>2 h</td>
<td>25.76 (2.78)</td>
<td>23.38 (3.51)</td>
<td>23.69 (1.46)</td>
<td>27.91 (4.58)</td>
<td>21.23 (2.32)</td>
<td>16.92 (0.96)</td>
</tr>
<tr>
<td>4 h</td>
<td>19.41 (2.54)</td>
<td>14.08 (2.26)</td>
<td>23.43 (2.51)</td>
<td>18.01 (2.90)</td>
<td>15.13 (1.90)</td>
<td>17.79 (1.35)</td>
</tr>
<tr>
<td>6 h</td>
<td>23.72 (1.49)</td>
<td>21.39 (3.72)</td>
<td>31.76 (2.69)**</td>
<td>19.34 (1.66)</td>
<td>17.27 (3.29)</td>
<td>19.36 (2.06)</td>
</tr>
<tr>
<td>12 h</td>
<td>25.85 (2.07)</td>
<td>25.76 (2.08)</td>
<td>35.87 (2.71)**</td>
<td>29.65 (2.14)</td>
<td>25.43 (0.93)</td>
<td>29.01 (6.05)**</td>
</tr>
<tr>
<td><strong>ANOVA P-value</strong></td>
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<td>0.1060</td>
<td>&lt;0.0001</td>
<td>0.0658</td>
<td>0.1663</td>
<td>0.0075</td>
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</table>

Data represented are mean (S.E.M.) of ΔMFI BLyS (n = 9). Differences between the basal expression and after several times of culture with each stimuli (P < 0.1, **P < 0.01, ***P < 0.005) were analysed by paired t-test. n.s.: non-significant; BLyS: B lymphocyte stimulator; SLE-ICs: immune complexes from SLE patient serum.

**Fig. 1** Influence of cytokines and SLE immune complexes on BLyS expression by healthy monocytes

Monocytes from healthy donors were incubated at different time points with cytokines (TNF-α, IFN-α, IFN-γ, GM-CSF) or immune complexes from SLE sera (SLE-ICs). Cultured cells were recovered, stained extra- and intracellularly for BLyS and analysed by flow cytometry. (A) Kinetics of surface:intracellular BLyS MFI ratio on monocytes after treatment with different cytokines or ICs or without any treatment. (B) BLyS levels (in ng/ml) in culture supernatant 6 h after cytokine or IC treatment. Graphs represent the mean (S.E.M.) obtained in nine independent experiments performed with different blood donors. Statistical differences between indicated treatments or with respect to untreated cells were evaluated by paired t-test. *P < 0.05, **P < 0.01, ***P < 0.005. BLyS: B lymphocyte stimulator; SLE-ICs: immune complexes from SLE patient serum; MFI: median fluorescence intensity.

### Rapid BLyS mobilization under IFN-α treatment in SLE monocytes

Our results indicated that IFN-α, and to a lesser extent SLE-ICs, induced de novo intracellular synthesis of BLyS in healthy monocytes that was further mobilized to the membrane and shed from the cell surface. Thus, to investigate whether these stimuli may be involved in the amplification of BLyS release in SLE, we analysed the kinetics of IFN-α induction at both the cellular and soluble levels in monocytes from SLE patients and healthy controls. To determine the possible influence of SLE-ICs, cultures...
Healthy and SLE monocytes were recovered after several hours of incubation with IFN-α and extra- and intracellular BLyS expression was analysed by flow cytometry. 

**A** Time course of extra- and intracellular BLyS MFI on healthy and SLE monocytes after treatment with IFN-α. 

**B** BLyS stacked levels in culture supernatant of unstimulated or IFN-α-treated SLE monocytes. Bars represent the mean (s.e.m.) of BLyS (ng/ml) from independent experiments performed with 9 blood donors and 16 SLE patients. Statistical differences between different times were evaluated by analysis of variance and Tukey’s post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001.

BLyS: B lymphocyte stimulator; MFI: median fluorescence intensity.

were performed with cells from patients presenting with low and high anti-dsDNA titres (groups were established on the basis of the median value: 78.00 U/ml) (Fig. 2A). Interestingly, extracellular BLyS levels before stimulation (t = 0) tended to be higher in patients with anti-dsDNA<sup>high</sup> titres [26.61 (s.e.m. 4.39)] compared with controls [17.67 (s.e.m. 2.00); P = 0.0529, t-test] and with the other patient group [14.86 (s.e.m. 4.30); P = 0.0801, t-test]. After IFN-α treatment (Fig. 2A), SLE monocytes did not show the noteworthy increase in intracellular BLyS expression, which peaked at 2 h, exhibited by controls. Accordingly, extracellular BLyS kinetics in patients were also altered. Thus the maximal surface expression observed at 6 h in controls became 4 h in anti-dsDNA<sup>low</sup> and 2 h in anti-dsDNA<sup>high</sup> SLE patients, probably due to the increased basal level of intracellularly stored BLyS.

The analysis of soluble BLyS in culture supernatants (Fig. 2B) indicated an accelerated release of this cytokine from monocytes of patients with anti-dsDNA<sup>high</sup> levels, since the highest levels previously observed in controls after 6 h (Fig. 1B) were even greater at the beginning of the culture (2 h) in these patients; the kinetics of BLyS release in the other group, however, was slower and similar to controls.

These data suggest faster IFN-α-induced BLyS mobilization from cytoplasm to membrane and subsequent secretion in monocytes from SLE patients, which could be accelerated depending on the levels of anti-dsDNA. Thus anti-dsDNA titres and disease activity scores were positively correlated in these patients (r = 0.666, P = 0.005; Pearson’s rank correlation test). Moreover, anti-dsDNA<sup>high</sup> SLE patients presented with higher IFN-α serum levels than anti-dsDNA<sup>low</sup> (36.29 (s.e.m. 24.29) vs 3.24 (s.e.m. 0.44), n.s.; t-test). Therefore the observed differences between monocytes from patients with high and low anti-dsDNA titres could be due to disease activity effects on monocyte activation.

**BLyS expression by peripheral blood cell subsets from SLE patients depends on disease activity**

In view of previous results and since anti-dsDNA and IFN-α serum titres have been commonly related to SLE activity, we considered it relevant to analyse BLyS expression by fresh cells from patients with different disease activity scores. To this end, the relative surface and intracellular protein expression levels of BLyS were determined by flow cytometry in pDCs, mDCs, B cells, neutrophils and monocytes from whole blood samples in 16 healthy donors and 23 SLE patients (see supplementary Fig. S1, available at Rheumatology Online). In SLE patients, a significantly higher expression of BLyS was found in the intracellular amount of mDCs and pDCs compared with healthy controls (Table 3). Moreover, B cells from patients presented increased BLyS protein levels compared with controls on their surface as well as intracellularly. Conversely, no significant differences in BLyS expression were found when comparing monocytes and neutrophils from patients and controls.

Next, BLyS expression was evaluated in patients grouped by disease activity as active (BILAG score ≥ 5, median anti-dsDNA titre 171.00 U/ml) and non-active SLE (BILAG score < 5, median anti-dsDNA titre 49.54 U/ml). Interestingly, active SLE patients displayed increased levels of surface BLyS expression on mDCs (0.94 vs 0.50, P = 0.0483) and monocytes (1.26 vs 0.88, P = 0.0337) as well as a reduced level of intracellular BLyS expression in monocytes and a similar but non-significant trend in mDCs (monocytes: 8.97 vs 15.12, P = 0.0457, mDCs: 6.72 vs 10.03, P = 0.1731) compared with non-active patients (Table 3). These results support a faster mobilization of BLyS from intra- to extracellular compartments in patients with active disease, as...
Data are presented as median (interquartile range) of AMFI BLyS. Differences between pairs of groups were analysed by Mann–Whitney U test. Groups of patients vs controls: *P < 0.05, **P < 0.01. Active vs non-active patients: †P < 0.05. BLyS: B lymphocyte stimulator; mDC: myeloid dendritic cells; pDC: plasmacytoid dendritic cells.

**Discussion**

A large body of research from studies of both the murine system and patients suggests a central role of BLyS overproduction in the pathogenesis of SLE [28–30]. Today it is known that this cytokine can be produced by myeloid cells as well as by activated B and T lymphocytes [37, 39]. However, the stimuli responsible for their enhanced production in SLE are not understood. Our in vitro results revealed that the expression of this molecule in both healthy and SLE monocytes was significantly influenced by the presence of IFN-α, a cytokine associated with the aetiopathology of SLE.

On the one hand, healthy monocytes showed an increase in intracellular BLyS levels after short-term treatment with IFN-α, and to a lesser extent with SLE-ICs, which was progressively mobilized to the cellular surface and finally released as soluble BLyS (Fig. 1). In accordance with our results, induction of BLyS expression has been described in myeloid cells, such as DCs and macrophages, after in vitro stimulation with type I IFNs [37, 47–50] and following IFN-α administration in murine models [51]. Also, treatment of SLE patients with an anti-IFN-α mAb reduces BLyS expression [52], whereas it has been reported that patients with multiple sclerosis under IFN-β therapy develop high BLyS serum levels, probably explained by the induction in myeloid cells [53]. Also, the up-regulatory effect of SLE-ICs containing nucleic acids on BLyS expression could be through triggering IFN-α production in these populations [54]. On the other hand, cellular BLyS expression after IFN-α treatment in monocytes from SLE patients showed differences with respect to controls, which may be due to the increased basal levels. Therefore SLE monocytes did not demonstrate a significant IFN-α-mediated de novo synthesis of BLyS, but showed an accelerated mobilization of this molecule to the plasma membrane and consequently earlier release, which was especially fast in SLE patients with anti-dsDNA high titres.

In agreement with this observation, it has been reported that soluble BLyS levels in pathological situations may be a consequence of its secretion from intracellular stores rather than de novo synthesis [55]. Moreover, since the anti-dsDNA serum titres of analysed patients were closely related to BILAG scores, our results could indicate a central role of disease activity in BLyS induction and mobilization to the cellular surface until finally being released extracellularly. Accordingly, serum levels of this cytokine have been positively correlated with anti-dsDNA antibodies and with SLEDAI scores in lupus patients with systemic manifestations [33, 56]. In fact, treatment with anti-BLYS mAb (phase III trials) in SLE has demonstrated more clinical benefit in patients with the most disease activity and high anti-dsDNA antibody or low complement levels [35, 57]. Furthermore, IFN-α levels have been associated with disease activity in SLE patients [10, 11], and Brkic et al. [58] reported an IFN type I activity associated with disease activity and BLyS expression in monocytes...
from patients with SS. Our in vitro results allow us to hypothesize that SLE monocytes, mainly those from active and/or anti-dsDNA^high^ patients, possess an IFN-\(\alpha\) activity, leading to increased BLYS production that was rapidly mobilized to the cellular surface. The ex vivo analysis of BLYS expression by fresh cells from SLE patients with different BILAG scores supports this hypothesis. First, our findings considerably extend previous studies reporting increased BLYS expression by mDCs, pDCs and B cells in SLE patients [36, 59] and suggest widespread overactivation of the immune system rather than implicating a single cell population for the BLYS pathological increase. However, when patients were grouped by disease activity, an interesting pattern emerged: as expected from previous studies [59, 60], monocytes and mDCs from active patients showed increased expression of surface-bound BLYS, but conversely, their level of intracellular expression was significantly reduced. Therefore monocytes and mDCs from patients with active disease presented increased relative surface vs intracellular BLYS levels compared with non-active patients, supporting the previously proposed mobilization of BLYS from intra- to extracellular compartments. Moreover, the ratios between intra- and extracellular BLYS expression correlated positively with the BILAG index [46], an effect that, to our knowledge, has not been described for any cell type so far.

The use of BLYS levels as a biomarker for disease activity has proved to be difficult, since the rate of production, release from the cell surface and consumption of circulating protein are potential confounders. Nevertheless, disease activity has been correlated with mRNA and surface protein BLYS levels in peripheral blood B cells [36, 37] and, more recently, with circulating BLYS concentration [31]. Although membrane-bound BLYS has potential biological effects, the soluble protein appears to mediate much of the function of this cytokine [40]. It has been reported that neutrophils could secrete BLYS from both the surface and the intracellular pool [42], but in other myeloid cells, such as monocytes or DCs, the release of BLYS depends on enzymic processing of the membrane-bound protein [38, 55], regulated by the presence of different pro-inflammatory cytokines [55, 61] as well as by cellular differentiation [62, 63]. Subsequently, increased BLYS expression and mobilization from intra- to extracellular compartments could lead to a continuous cellular release, leading to the increased serum levels usually detected in SLE patients. Moreover, the elevated IFN-\(\alpha\) production identified in a large group of SLE patients [6, 64] can induce the differentiation of normal monocytes into mature DCs [65, 66], thus enhancing BLYS gene expression and protein release and the consequent autoreactive B and T cell responses, resulting in higher autoantibody production [9]. Finally, the SLE-ICs containing nucleic acids trigger the production of high levels of IFN-\(\alpha\) [54], thus likewise increasing BLYS release.

Taken together our observations suggest that anti-dsDNA, IFN-\(\alpha\) and BLYS are acting in a vicious cycle, with BLYS leading to increased autoantibody production, forming SLE-ICs that induce IFN-\(\alpha\) production and consequently increased BLYS production, mobilization to the membrane and release. This picture supports the observed correlation between BILAG score and BLYS membrane mobilization in monocytes from active patients.

In conclusion, the findings of this study reveal that BLYS induction and mobilization in monocytes are significantly influenced by IFN-\(\alpha\) and SLE-ICs. In addition, BLYS

![Fig. 3 BLYS expression by fresh cells from SLE patients in disease activity](https://academic.oup.com/rheumatology/article-abstract/53/12/2249/1803392)

(A) Ratio between extra- and intracellular BLYS levels in blood cell subsets from active and non-active SLE patients. Scatter plots represent the ratio between surface and intracellular ΔMFI BLYS levels from active and non-active SLE patients and healthy donors (control). Horizontal bars show the median. Statistical significance was assessed by Mann–Whitney U test. (B) Correlation of BLYS expression in monocytes and SLE disease activity. The correlation between the extra-/intracellular BLYS ratio ΔMFI and BILAG index was calculated using the Pearson’s rank correlation test. BLYS: B-lymphocyte stimulator; MFI: median fluorescence intensity; mDC: myeloid dendritic cells; pDC: plasmacytoid dendritic cells.
expression in SLE patients supports the existence of general-ized immune stimulation, since there is not a single cell population responsible for the increased systemic BLyS levels. Specifically, monocytes and mDCs from active patients presented the highest ratio between membrane-bound and intracellular BLyS expression, not previously described for any cell type. Therefore patients with higher levels of IFN-α and/or anti-dsDNA titre may benefit most from BLyS blocking therapy.

**Rheumatology key messages**

- Monocyte B-lymphocyte stimulator induction and mobilization are significantly influenced by IFN-α and SLE immune complexes.
- Several blood cells are responsible for B-lymphocyte stimulator overexpression in SLE, especially monocytes and myeloid dendritic cells.
- Patients with high IFN-α and/or anti-dsDNA levels may benefit most from B-lymphocyte stimulator blocking therapy.

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**Supplementary data**

Supplementary data are available at Rheumatology Online.

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BlyS expression and mobilization in SLE


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