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Basic science

SLE stratification based on BAFF and IFN-I bioactivity for biologics and implications of BAFF produced by glomeruli in lupus nephritis

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Abstract

Objective: B-cell activating factor (BAFF) is implicated in SLE pathogenesis. Blocking BAFF signalling has contributed to reducing glucocorticoid dosage and preventing organ damage. However, clinical characteristics of patients who may benefit from this therapy are not yet fully elucidated. Therefore, we identified patients with high BAFF-bioactivity to investigate their clinical characteristics and BAFF-producing cells.

Methods: We established the reporter cell for BAFF and investigated the clinical characteristics of SLE patients with high BAFF-bioactivity. We identified BAFF-expressing kidney cells using publicly available scRNA-seq data and immunohistological analysis. SLE patients were stratified based on the bioactivity of BAFF and type-I IFN (IFN-I) to identify associated characteristic clinical manifestations.

Results: SLE patients, especially patients with LN, had significantly higher serum BAFF-bioactivity than healthy controls (HC) and non-LN patients. Additionally, single-cell-RNA-seq data and immunohistological analysis of kidney samples from LN patients revealed that BAFF is expressed in glomerular macrophages and mesangial cells. Notably, BAFF bioactivity was elevated in the urine of LN patients compared with that of non-LN patients, while no IFN-I bioactivity was detected in the urine. Furthermore, SLE stratification based on bioactivities of serum BAFF and IFN-I revealed the clinical characteristics of patients: high BAFF represented patients with LN and high IFN-I represented patients with blood and skin manifestations.

Conclusions: Monitoring urinary BAFF-bioactivity may be valuable in diagnosing LN. Furthermore, stratification based on serum BAFF and IFN-I bioactivities may allow the identification of appropriate patients for biologics targeting BAFF and IFN-I.

Keywords: SLE, B-cell activating factor (BAFF), LN, glomerular macrophages, IFN-I

Rheumatology key messages

- SLE stratification based on BAFF and IFN-I biological activity revealed clinical characteristics of SLE patients.
- BAFF bioactivity is elevated in serum and urine of patients with lupus nephritis.
- BAFF produced by glomerular macrophages and mesangial cells causes lupus nephritis pathogenesis.

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Introduction

SLE is an autoimmune disease that affects various organ systems in different individuals, including the central nervous system, immune system, hematopoietic and haemostatic system, skin, kidney, and musculoskeletal system [1]. Among these, LN is a major organ disorder that affects 40% of adult SLE patients, leading to end-stage renal dysfunction (ESRD) in some patients despite intense treatments [2].

Although the exact cause of SLE remains unknown, autoantibodies, due to the disruption of immune tolerance to nucleic acids and self-antigens, are vital in SLE pathogenesis [3]. B cells originate from lymphoid progenitors in the bone marrow, are mobilized into the blood as naïve B cells, and migrate to the germinal center (GC) to differentiate into mature B cells, where immunoglobulin class-switch and affinity maturation of B cell receptors (BCR) occur to produce antibodies with high affinity and function [4]. Peripherally, an increase in short-lived plasmablasts and B cells with an unclass-switched memory phenotype [5], a decrease in B cells with somatic mutant BCR, and a linkage of the BCR repertoire between activated B cells with a naive phenotype and antibody-producing cells (APCs) have been reported in SLE [6]. Additionally, a new B-cell population that neither expresses IgD nor CD27 (double-negative B cell: DNB) is reportedly increased in the periphery of SLE [7, 8]. These cells express a gene set similar to mouse Age-Associated B cells (ABC), including *ITGAX* (CD11c) and *TBX21* (T-bet) [9, 10]. They are reportedly differentiated from naive B cells upon innate immune stimulation, such as toll-like receptor 7 (TLR7) and BCR. They also express the low GC-homing C-X-C chemokine receptor type 5 (CXCR5) and high peripheral tissue-migratory CC motif chemokine receptor 9 (CCR9) and CXCR3 [7, 11]. Therefore, these APCs are thought to differentiate in an extrafollicular fashion [12]. Recently, the existence of $CD11c^{hi}T\text{-}bet^{+}$ and activated B cells expressing a gene set related to ABCs has been reported, suggesting that B cells in the kidneys may play roles in LN pathogenesis [11, 13].

B-cell activating factor (BAFF), belonging to the TNF family, is known to promote B-cell survival and differentiation via BAFF Receptor (BAFFR), B-cell maturation antigen (BCMA), and transmembrane activator and CAML interactor (TACI) [14], and may contribute to DNB cell differentiation [7]. The involvement of BAFF in SLE pathogenesis [15–17] has been demonstrated in murine models [18]. Furthermore, the anti-BAFF antibody belimumab has been effective in treating SLE and is approved by the FDA as the first biologic for SLE [19, 20]. Recently, the addition of belimumab to standard initial therapy for LN was shown to improve renal dysfunction [21]. However, the efficacy of belimumab treatment was only ∼30% in a clinical trial [19]. Among these patients, those with high serum BAFF levels were determined to respond to belimumab treatment by *post hoc* analysis [22]. However, BAFF concentrations by ELISA varied [23–25], and the responder cut-off value was within the normal range of healthy donors [22]. Furthermore, the clinical profile of patients who respond well has not yet been elucidated. Therefore, we attempted to develop a cell-based assay system to monitor BAFF biological activity and identify the patient profiles in which BAFF activity is elevated. Similarly, type I IFN (IFN-I) is involved in SLE pathogenesis [26, 27]. Anifrolumab, an interferon-alpha/beta receptor 1 (IFNAR1) antibody, has been approved by the FDA for SLE for its efficacy [28]. However, only those patients with high ISG signature respond to anifrolumab [29]. The appropriate patient profile for this biologic has not yet been determined. Therefore, we attempted to stratify SLE patients with high levels of BAFF and IFN-I biological activities and determine the clinical profile of these patients.

Methods

Clinical samples

We included patients with SLE admitted to the Department of Clinical Immunology, Osaka University, from 2012–2021. The enrolment of all participants was approved by the Institutional review board of Osaka University (12456–3 and 11122–4). All participants gave written informed consent in accordance with the Declaration of Helsinki. SLE diagnoses were based on the 1997 ACR revised criteria for the classification of SLE [30] and/or Systemic Lupus International Collaborating Clinics (SLICC) criteria [31]. Healthy control (HC) samples were obtained from 43 volunteer donors. Patients with infections or cancers were excluded from this study. Disease activity of SLE was assessed according to the Safety of Estrogens in Lupus Erythematosus National Assessment SLEDAI (SELENA SLEDAI) score [32]. The histological classification was determined according to the International Society of Nephrology/Nephrological Pathology (ISN/RPS) [33]. Chronic organ damage was evaluated using the SLICC/ACR Damage Index (SDI) [34]. Serum was collected from enrolled subjects, and patient samples were collected at the time of admission or prior to the start of treatment, and one sample of each individual were measured. Urine samples were collected and evaluated at any time from patients whether they were hospitalized or outpatient.

Establishment of reporter cell for BAFF

The BAFFR–BCMA chimeric receptor was designed by connecting the extracellular region of BAFFR and the transmembrane and intracellular region of BCMA with a linker sequence. BAFFR–BCMA chimeric construct was generated by overlapping PCR and fused into EF1a-EGFP Vector (Vectorbuilder, IL, USA) (see Supplementary Data S1, available at *Rheumatology* online). The expression vector of BAFFR–BCMA chimeric receptor was lentivirally transduced into HEK-Null1 reporter cells that produce secreted embryonic alkaline phosphatase (SEAP) following nuclear factor-kappa B (NF-κB) activation. A single clone was obtained by limiting dilution.

Cell-based reporter assay

HEK-Blue IFN-α/β Cells and HEK-Blue Null1 cells were purchased from InvivoGen. For measurement of BAFF or IFN-I bioactivity, each reporter cell was incubated for 24 h with 20% serum or urine. SEAP in the supernatant was detected with QUANTI-Blue (InvivoGen, CA, USA) and quantified by measuring the optical density of the sample at 620 nm.

Single-cell RNA-seq analysis

Raw and normalized count matrices of the single-cell RNA-seq data were downloaded from Immport [35]. After filtering, cell type annotation, and removal of keratinocytes and skin fibroblasts, PCA and UMAP were performed to analyse kidney-derived cells. The expression level of the BAFF-encoding TNF superfamily member 13 b (*TNFSF13B*) gene in

the renal cells derived from the SLE patients was visualized based on kernel density estimation as implemented in the Nebulosa package (see Supplementary Data S1, available at *Rheumatology* online).

Immunohistological analysis

After deparaffinization, antigen activation and blocking, the renal biopsy specimens were stained with primary antibodies for BAFF (LS-B2081, LSBio, WA, USA), CD68 (375602, Biolegend, CA, USA) and αSMA (NB300-978, Novus Biologicals, CO, USA) and secondary antibodies (see Supplementary Data S1, available at *Rheumatology* online). The specimens were observed by confocal microscopy (Olympus FV3000, Tokyo, Japan).

Statistical analysis

All statistical analyses were conducted using JMP Pro 14.0.0. Non-parametric Mann– Whitney *U* or Steel–Dwass tests were used to compare two groups or multiple pairs, respectively. *P*-values <0.05 were considered statistically significant. The correlation between clinical parameters and BAFF level was determined using Spearman's rank correlation coefficient (ρ).

Results

Development of reporter cells that enable monitoring the biological activity of BAFF

To monitor the biological activity of BAFF, we first transduced BAFFR- or BCMA-expression vector to HEK-Blue Null1 cells that have the IFN-γ minimal promoter and five NF-κB and AP-1 binding sites, followed by the secreted embryonic alkaline phosphatase (*SEAP*) reporter gene. However, BAFFR-expressing cells did not react with BAFF. Because BCMA is a receptor for BAFF and its homologue, a proliferation-inducing ligand (APRIL) [36], we constructed a BAFFR–BCMA chimeric receptor by combining the extracellular and transmembrane regions of BAFFR with the intracellular region of BCMA to specifically respond to BAFF (Fig. 1A). The BAFFR–BCMA-chimeric receptor-expressing vector was introduced into HEK-Blue Null1 cells (Supplementary Fig. S1A, available at *Rheumatology* online). The reporter cells (BAFFR-BCMA-HEK-Blue) responded specifically to recombinant BAFF in a concentration-dependent manner and not to APRIL or other cytokines (Fig. 1B; Supplementary Fig. S1B and C, available at *Rheumatology* online), indicating that BAFFR-BCMA-HEK-Blue cells respond specifically to BAFF.

Elevated serum BAFF bioactivity in patients with LN

In this study, 105 SLE patients and 43 healthy volunteers (HC) were enrolled (the participant profiles are listed in Table 1). The BAFF biological activity in sera was measured in BAFFR-BCMA-HEK-Blue cells. BAFF-bioactivity was significantly higher in SLE sera than in HC sera (Fig. 1C). There was an age and gender bias between the two groups. However, there was no correlation between BAFF bioactivity and age (Supplementary Fig. S2A, available at *Rheumatology* online); BAFF bioactivity was higher in males than in females (Supplementary Fig. S2B, available at *Rheumatology* online). Therefore, we considered that the BAFF bioactivity was elevated in SLE serum compared with that in HC. Then, clinical characteristics of SLE patients with high BAFF-bioactivity were investigated from clinical records. Serum BAFF-bioactivity slightly correlated with SLE disease activity as evaluated by the SELENA SLEDAI score (Fig. 1D) [32], but there was no correlation with SLEDAI in the high-BAFF activity group. Serum BAFF bioactivity was also increased in the presence of LN (Fig. 1E), whereas it was not correlated with anti-double strand DNA (dsDNA) antibody titre and serum complement 3 (C3) value (Supplementary Fig. S3A and S3B, available at *Rheumatology* online). Consistent with the previous report [22], serum BAFF-bioactivity did not correlate with *TNFSF13B* mRNA expression in peripheral blood mononuclear cells (PBMCs) from SLE patients (Supplementary Fig. S3C, available at *Rheumatology* online). Subsequently, we investigated the relevance between BAFF-bioactivity and the histological classification of the International Society of Nephrology/Nephrological Pathology (ISN/RPS) [33]. To this end, we enrolled 21 SLE patients whose serum was stored at the same time as the renal biopsy. Serum BAFF-bioactivity tended to be higher in LN class III or IV patients (Fig. 1F). These results indicate that monitoring the biological activity of serum BAFF is valuable and that its level is closely related to the presence of LN.

Expression of BAFF in glomerular macrophages and mesangial cells in LN

Next, we investigated the expression of BAFF in the kidneys of patients with LN. First, we accessed open-source AMP-SLE scRNA-seq data from the kidneys and skin of LN patients [35]. We analysed raw data and normalized count matrices of Fluidigm C1-based scRNA-seq data that met several criteria (Supplementary Fig. S4A, available at *Rheumatology* online). After annotation of cell types (Supplementary Fig. S4B, available at *Rheumatology* online) [37], data for keratinocytes and fibroblasts from the skin were excluded, and only cells originating from the kidneys were analysed (Fig. 2A). *TNFSF13B* was expressed in renal leukocytes and activated mesangial cells (Fig. 2B). Next, we evaluated open-source AMP-SLE scRNA-seq data obtained from PBMCs of SLE patients [13]: macrophages predominantly expressed *TNFSF13B* (https://singlecell.broadinstitute.org/single_cell/study/SCP279/amp-phase-1) (Supplementary Fig. S4C, available at *Rheumatology* online). Because macrophages in lupus kidneys are derived from blood circulation [13], the renal biopsy specimens of active LNs were immunohistologically investigated, and we confirmed the expression of BAFF in CD68-positive infiltrating macrophages and α-SMA-positive activated mesangial cells (Fig. 2C; Supplementary Fig. S5, available at *Rheumatology* online). Furthermore, we analysed the expression of receptors of BAFF in the kidneys of LN patients by accessing the open-source AMP-SLE scRNA-seq data. Although the number of analysed cells was extremely small to obtain definitive results, *TNFRSF13C* (BAFFR) and *TNFRSF13B* (TACI) were detected in a subset of activated B cells (CB0) that also express *ITGAX* (CD11c) and *TBX21* (T-bet). *TNFRSF17* (BCMA) was detected in a subset of plasmablasts (CB1) (Supplementary Fig. S6, available at *Rheumatology* online). These results suggest that BAFF produced by glomerular macrophages and mesangial cells may activate local B cells and exacerbate LN.

Increased BAFF bioactivity in the urine of patients with LN

Therefore, considering the possibility of detecting BAFF in the urine of patients with LN, we collected urine and serum

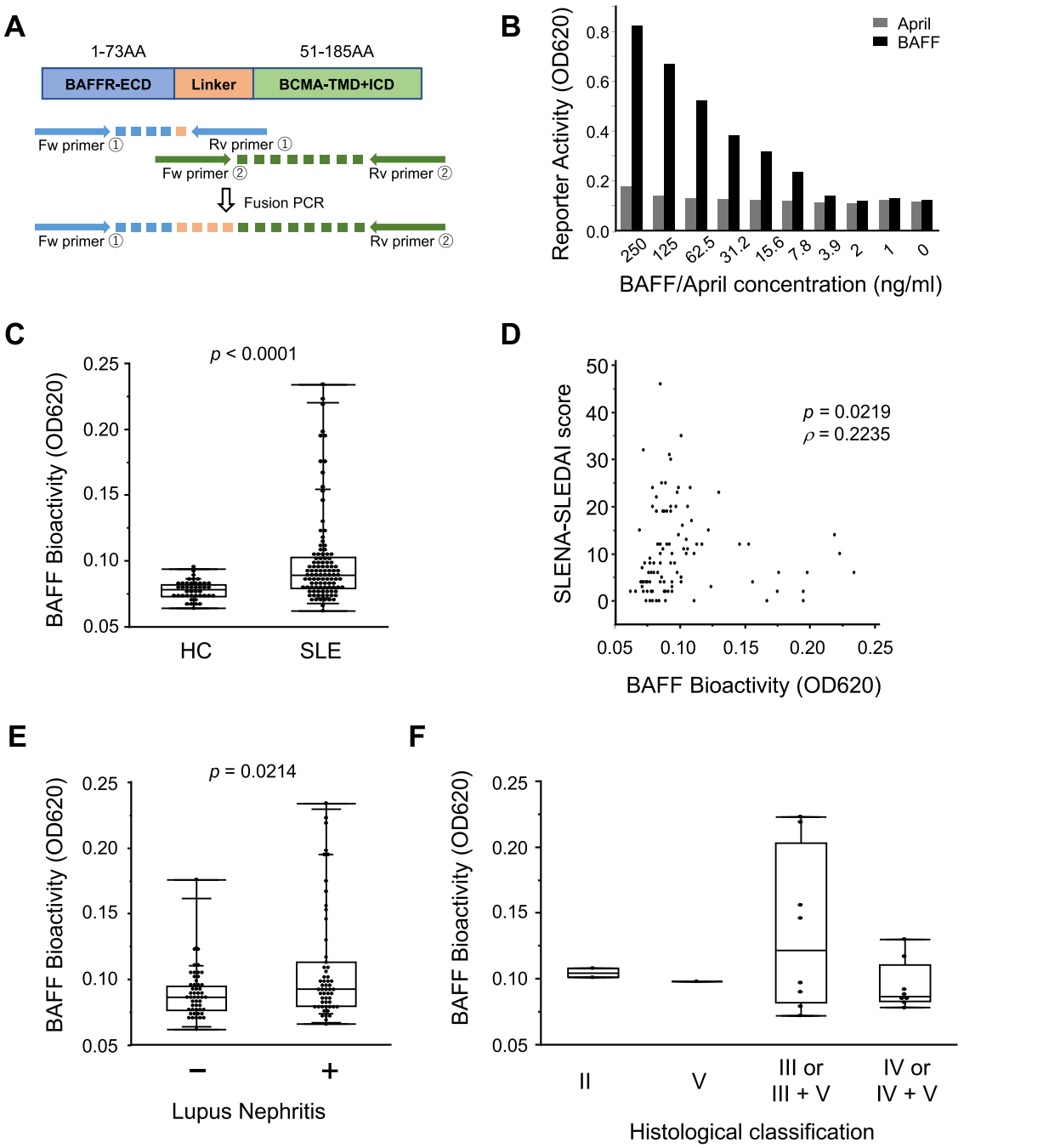

Figure 1. Development of reporter cells for BAFF bioactivity and increased serum BAFF bioactivity in patients with LN. (**A**) Design of BAFFR–BCMA chimeric receptor. (**B**) Dose-dependent reaction to recombinant BAFF by Reporter cells expressing BAFFR–BCMA chimeric receptor. (**C**) Serum BAFF bioactivity in SLE ($n = 105$) and HC ($n = 43$) measured by reporter cells for BAFF. (**D**) Correlation between serum BAFF bioactivity and SLE disease activity, assessed according to SLEDAI-2K ($n = 105$). (**E**) Serum BAFF bioactivity and LN. Patients with SLE were divided into two groups according to the presence ($n = 55$) or absence ($n = 50$) of LN. (**F**) Serum BAFF bioactivity and histopathological features of LN. Serum was measured on the same day as the renal biopsy ($n = 21$). Renal histology was evaluated by the histological classification of the International Society of Nephrology/Nephrological Pathology (ISN/RPS). Statistical analysis was performed using Mann–Whitney U (**C**, **E**), Spearman correlation (**D**), and Steel–Dwass tests (**F**). The box chart indicates the 75th percentile (upper), median (middle), and 25th percentile (lower)

samples from 47 patients with SLE with or without LN, 11 patients with nephritis due to ANCA-associated vasculitis (AAV), and 11 healthy donors (Supplementary Table S1, available at *Rheumatology* online). Urinary BAFF-bioactivity in SLE patients was significantly higher than that in healthy controls (Fig. 3A). In particular, it was significantly higher in LN patients than in non-LN patients and patients with AAV-related nephritis (Fig. 3B), suggesting that BAFF detected in the urine may be derived from glomerular macrophages or mesangial cells. In contrast, IFN-I bioactivity could not be detected in the urine of these participants (Fig. 3C), and the ratio of urine to serum BAFF bioactivity was significantly higher than the ratio of IFN-I bioactivity (Fig. 3D). Subsequently, the ROC curve was plotted to evaluate AUC and determine the cut-off value of the urinary BAFF bioactivity, which can eventually be used to diagnose LN. Converting

Table 1. Characteristics of patients with SLE and HC

Characteristics and clinical manifestations		SLE ($n = 105$)	HC ($n = 43$)
Age (years)		41 (34–49)	34 (32–38)
Sex (female)		97 (92.4)	14 (32.6)
Duration of disease (years)		7 (1–16)	
In-patient		63 (60)	
SLEDAI-2K		8 (4–14)	
SDI		1 (0–2)	
PSL (mg/day)		7.5 (2–15)	
Immunosuppressive agents	IVCY	8 (7.6)	
	MMF	15 (14.3)	
	AZA	6 (5.7)	
	CyA	9 (8.6)	
	TAC	19 (18.1)	
	HCQ	24 (22.9)	
ANA		103(98.1)	
dsDNA antibody (IU/ml)		26.5 (3.8–75.8)	
Anti-Sm Antibody		44 (41.9)	
C3 (mg/dL)		65(47–85)	
CRP (mg/dL)		0.09 (0.04–0.38)	
UPCR		1.09 (0.25–2.623)	
Rash		21 (20)	
Oral ulcers		7 (6.7)	
Arthritis		21 (20)	
Serositis		16 (15.2)	
Nephritis		55 (52.4)	
NPSLE		3 (2.9)	
Vasculitis/Retinopathy		6 (5.7)	
Leukopenia (<4000/μl)		13 (12.4)	
Lymphopenia (<1500/μl)		90 (85.7)	
Thrombocytopenia (<100 000/μl)		12 (11.4)	
Diagnosis of antiphospholipid syndrome		19 (18.1)	

Data are presented as n (%) or median (IQR).
CyA: ciclosporin A; double-stranded DNA; HC: healthy control; IVCY: intravenous cyclophosphamide; NPSLE: neuropsychiatric SLE; PSL: prednisolone; SDI: SLICC/ACR Damage Index; TAC: tacrolimus; UPCR: urine protein to creatinine ratio.

the BAFF activity to its corresponding concentration values obtained from the SEAP production of recombinant protein (Supplementary Fig. S7, available at *Rheumatology* online) suggested that at least 48 ng/ml BAFF contributed to the diagnosis of LN (AUC 0.743) with a sensitivity of 63% and specificity of 94%. However, the accuracy was low owing to the limited number of cases (Fig. 3E). These results suggest that urinary BAFF bioactivity could be a urinary biomarker of LN.

Stratification of SLE based on BAFF and IFN-I bioactivity

Finally, serum bioactivity of BAFF and IFN-I in individual SLE patients was plotted on two axes to stratify SLE patients. Patients were divided into four groups: low BAFF, low IFN-I; high BAFF, low IFN-I; low BAFF, high IFN-I; and high BAFF, high IFN-I. Notably, patients with high BAFF and IFN-I were rare, and patients with high BAFF or IFN-I were classified in an independent group (Fig. 4A). Furthermore, the clinical characteristics of patients who exhibited high bioactivity of BAFF or IFN-I were analysed. Patients with high serum BAFF-bioactivity were significantly associated with LN, whereas those with high IFN-I-bioactivity were associated with leukopoenia, low complement levels, rash, arthralgia and serositis (Table 2; Supplementary Fig. S8, available at *Rheumatology* online). Retrospective analysis of clinical records showed that patients in high BAFF or IFN-I populations had received intensified treatment, represented by the increased dosage of steroids and addition of immunosuppressive drugs (Fig. 4B). These results suggest that stratification based on serum BAFF and IFN-I bioactivity could be used to screen patients who will benefit from treatment with available biologics, such as belimumab and anifrolumab.

Discussion

In this study, by establishing a cell-based reporter assay system that can measure the biological activity of BAFF, we have identified a profile of SLE patients who exhibit high BAFF bioactivity in serum and urine. The serum level of BAFF bioactivity associated with the presence of LN and correlated with the corresponding SLEDAI. Additionally, the bioactivity of BAFF, but not IFN-I, was elevated in the urine of patients with LN, presumably due to the expression of BAFF in glomerular macrophages and mesangial cells. Furthermore, based on the bioactivity of serum BAFF and IFN-I, SLE patients were further classified into four groups, and the bioactivity of each cytokine was associated with clinical symptoms. Therefore, the stratification based on BAFF and IFN-I would allow the selection of SLE patients suitable for biologics targeting BAFF and IFN-I signalling.

Elevated BAFF concentrations in SLE serum have been reported; high BAFF concentration was suggested to be a valuable biomarker to predict belimumab responder [22]. However, its reported levels remain inconsistent [23–25]. For example, patients with serum BAFF concentrations of 2 ng/ml or higher are reportedly responsive to belimumab [38]. However, this cut-off concentration could be the normal range for healthy individuals [39]. Additionally, BAFF mRNA expression in PBMCs has been shown not to correlate with serum BAFF protein expression [22]. Thus, evaluation of BAFF mRNA does not appear to be helpful in stratifying SLE. Furthermore, the biological activity of BAFF varies depending on its oligomerization status (monomer < trimer < 60-mer). However, the ELISA method cannot accurately identify the oligomer of BAFF [40]. In contrast, cell-based reporter assay systems can directly assess the biological activity of BAFF and may be valuable in identifying those SLE patients whose BAFF levels contribute to the pathogenesis.

We revealed that SLE patients with LN exhibit higher serum bioactivity of BAFF than those without LN. Additionally, urine from patients with LN had higher bioactivity of BAFF and no bioactivity of IFN-I. However, the bioactivity of both cytokines in serum was elevated. This suggests that local BAFF produced by glomeruli may contribute to the exacerbation of LN. BAFF is reportedly produced by monocytes, macrophages, T cells, neutrophils, epithelial cells and synovial fibroblasts [14, 41–43]. By detailed open scRNA-seq data and immunohistological analysis, we confirmed that BAFF is produced by infiltrating glomerular macrophages and mesangial cells. Mesangial cells produce various cytokines that cause LN [44], and monocytes infiltrating the kidneys resemble inflammatory macrophages [13]. Thus, our findings are consistent with the previous reports that glomerular macrophages and mesangial cells produce BAFF.

A specific B-cell population, $CD27^{-}IgD^{-}$double negative (DN) B cells, is reportedly involved in SLE pathogenesis [7,

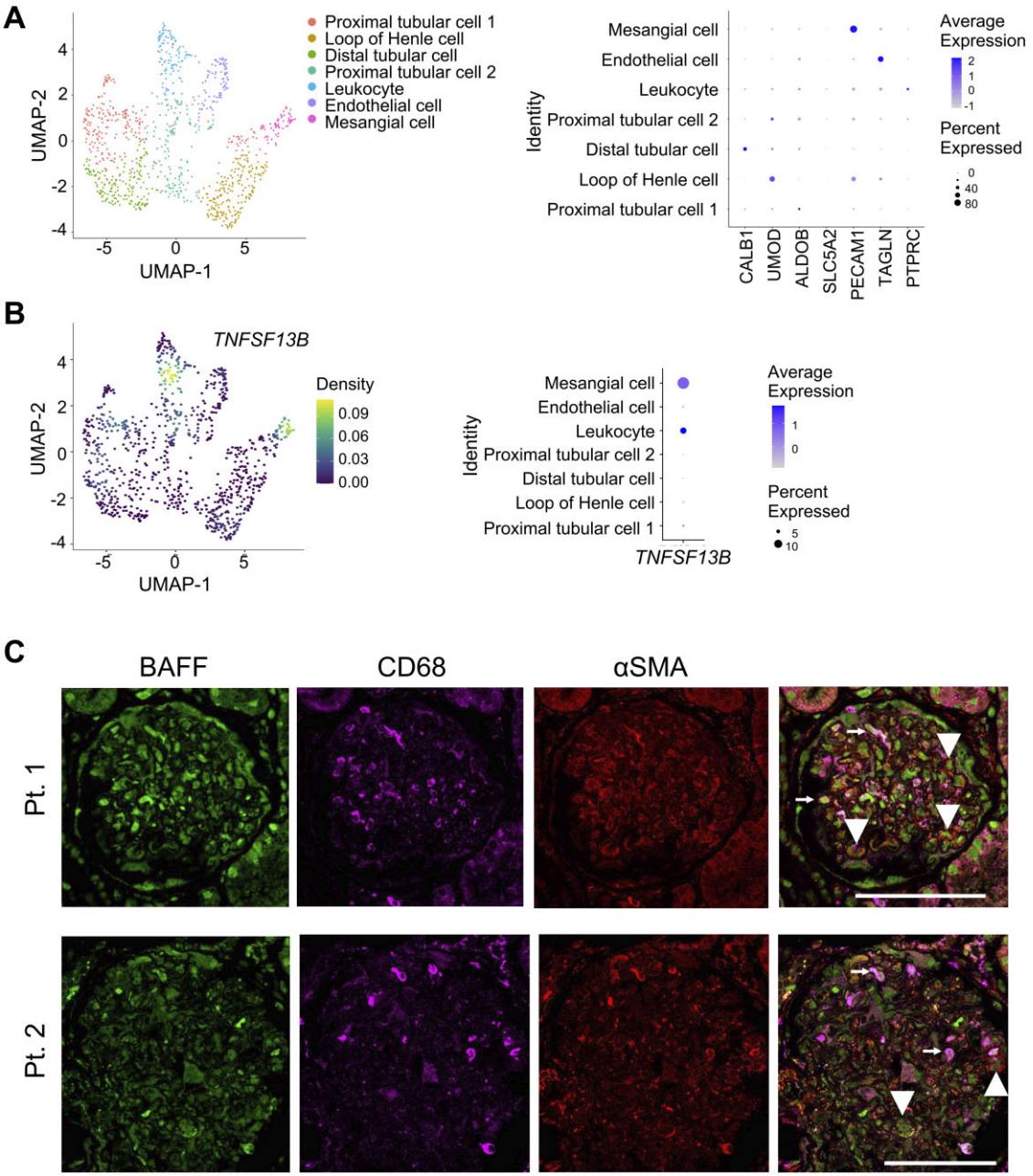

Figure 2. BAFF expression in glomerular macrophages and activated mesangial cells in the kidneys of patients with LN. (**A**, **B**) Expression of BAFF evaluated by scRNA-seq. Raw and normalized count matrixes of scRNA-seq data were analysed. Each cell population was annotated based on marker genes, and UMAP (left) and clustering (right) were visualized (**A**). The expression of the *TNFSF13B* (BAFF) in the renal cells (935 cells) derived from SLE patients was visualized based on kernel density estimation as implemented in the Nebulosa package (**B**). (**C**) Immunostaining of renal biopsy specimens of LN. BAFF (green), macrophage (magenta) and activated mesangial cells (red) in two different active LN specimens were stained by anti-BAFF, anti-CD68 and anti-αSMA antibodies, respectively (Scale bar: 100 μm). The small arrows and big arrowheads highlight the co-localization of BAFF with glomerular macrophages and mesangial cells, respectively

8]. DN B cells are thought to differentiate extrafollicularly, produce autoantibodies, and infiltrate peripheral organs. These cells are the human counterpart of aged B cells (ABCs) in mice because of similarity in the mRNA expression of *ITGAX* (CD11c), *TBX21* (T-bet), *CCR9* and *CXCR3* [9, 10]. Additionally, stimulation of B-cell receptors, TLR7, and BAFF is required for DN B-cell development [7]. Because activated B cells expressing the ABC cell gene set reportedly infiltrate the kidneys [13], we analysed the public scRNA-seq data from SLE kidneys. We observed that the mRNA expression of BAFF receptors, including *TNFRSF13C* (BAFFR) and *TNFRSF13B* (TACI), was expressed in CD11c- and T-bet-expressing cells; however, the cell numbers were small and inconclusive. Thus, BAFF is likely produced by glomerular macrophages and mesangial cells, allowing activation and survival of infiltrating B cells such as DN B cells.

Renal biopsy is the golden standard for diagnosing LN and is recommended to be performed to determine the appropriate treatment. In some cases, however, this procedure may not be possible because of the risk of bleeding, progressive renal dysfunction, or a patient's refusal to undergo a renal biopsy. Therefore, developing a biomarker to infer the histological

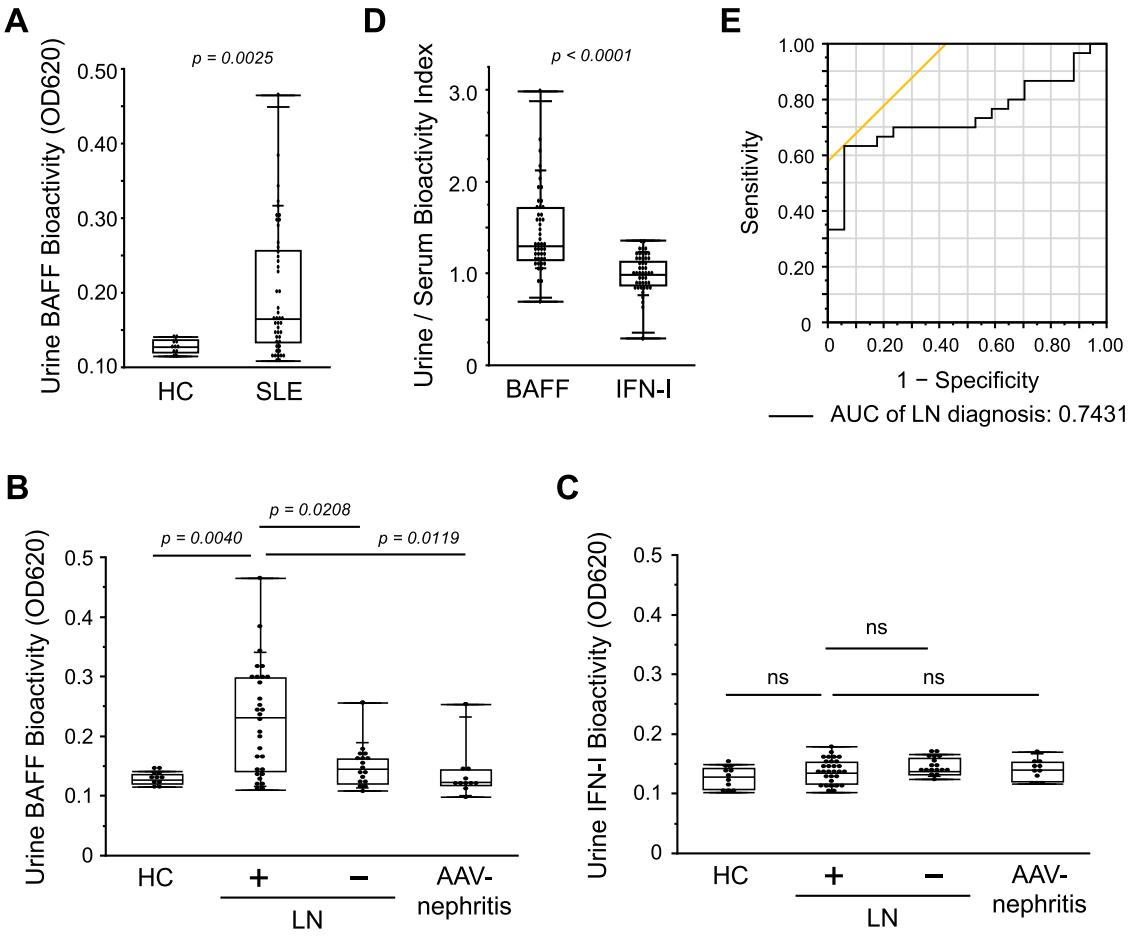

Figure 3. Increased urinary BAFF bioactivity in patients with LN. (**A**) Urinary BAFF bioactivity. BAFF bioactivity in the urine of patients with SLE ($n = 47$) and healthy volunteers (HC, $n = 11$) was measured by BAFFR–BCMA reporter cells. (**B**, **C**) Urinary BAFF and IFN-I bioactivities in LN. Bioactivity of BAFF (**B**) and IFN-I (**C**) in the urine of patients with LN [LN (+), $n = 30$], without LN [LN (–), $n = 17$], with AAV-related nephritis (AAV, $n = 11$), and healthy volunteers (HC, $n = 11$) were measured. (**D**) Ratio of BAFF or IFN-I bioactivity in urine to that in serum. (**E**) ROC curves for the presence of LN (line). BAFF concentrations were calculated by the bioactivity of recombinant BAFF as measured by reporter cells. Statistical analysis was performed using Mann–Whitney U (**A**, **D**) and Steel–Dwass tests (**B, C**). The box chart indicates the 75th percentile (upper), median (middle), and 25th percentile (lower)

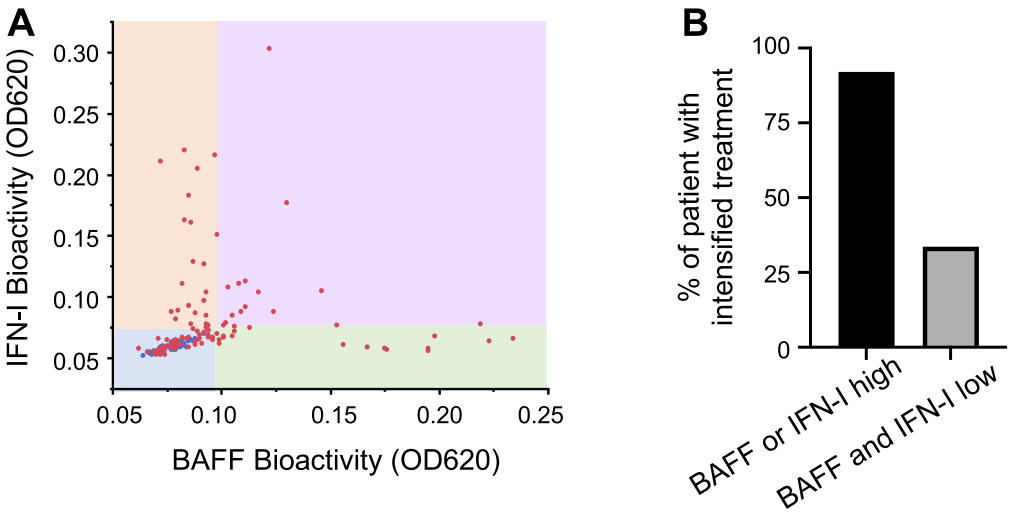

Figure 4. Stratification of SLE patients based on serum BAFF and IFN-I bioactivities. (**A**) The biological activity of serum BAFF and IFN- I in individual SLE patients is represented on two axes. The patients were divided into four groups according to the high and low bioactivity of BAFF and IFN-I. The cut-off level of serum BAFF and IFN-I was determined by the normal upper limit plus 1SD. (**B**) Percentage of patients requiring enhanced treatment. The percentage of SLE patients with increased treatment intensity was calculated among SLE patients with a high biological activity of either BAFF or IFN-I or low activity of both

Table 2. Clinical manifestations of SLE patients with high bioactivity of BAFF and IFN-I

Clinical manifestations	BAFF	IFN-I
Serositis ($n = 16$)	$P = 0.1399$	$\mathbf{P = 0.0006}$
Arthritis ($n = 21$)	$P = 0.1447$	$\mathbf{P = 0.0014}$
Rash ($n = 21$)	$P = 0.625$	$\mathbf{P = 0.0013}$
Leukopenia ($n = 7$)	$P = 0.9028$	$\mathbf{P = 0.0014}$
Low C3	$\rho = 0.0219$	$\boldsymbol{\rho = -0.5457}$
	$P = 0.8277$	$\mathbf{P < 0.0001}$
Diagnosis of Nephritis ($n = 55$)	$\mathbf{P = 0.0214}$	$P = 0.4545$

Manifestation of SLE is listed (n: number of symptomatic patients among 105 SLE patients). Data are presented as P-values for the correlation between the bioactivity of BAFF or IFN-I and the presence or absence of clinical symptoms in SLE, or correlation coefficient between the level of bioactivity of BAFF or IFN-I and serum C3. Statistical analysis was performed using Mann–Whitney U or Spearman correlation test. BAFF: B-cell activating factor. Bold text highlights significance.

type of LN is crucial. Many cytokines such as IL-6, TGF-β, TNF-α, and IFN-γ in urine have been validated as biomarkers for LN. However, none have been clinically applied due to the lack of significant results in large-scale validation and difficulty differentiating LN from urinary tract infection [45, 46]. In contrast, urinary BAFF bioactivity was not elevated during urinary tract infection, allowing us to distinguish those patients with LN. Although no definitive claims can be made, monitoring urinary BAFF-bioactivity may be valuable as a biomarker for diagnosing LN and aid in selecting optimal treatment strategies for those patients who cannot undergo a renal biopsy.

Recently, the FDA approved the anti-BAFF antibody belimumab and the anti-IFN antibody anifrolumab as anti-SLE drugs [19, 28]. Post-hoc analyses of clinical trials of these biologics have shown that belimumab is more effective in patients with high serum BAFF concentration [22]. Similarly, anifrolumab is more effective in patients with high ISG-expressing PBMCs [29], suggesting the importance of SLE stratification. In this study, we used a simple method that can be evaluated with the same serum sample, allowing stratification of SLE patients based on serum bioactivity of BAFF and IFN-I. BAFF is known to be produced by IFN-I stimulation [14, 47]. Thus, both IFN-I and BAFF are expected to be elevated in patients with SLE. However, patients with high serum IFN-I activity and those with high BAFF activity were found to differ from each other. Consistent with our observation from RNA-seq analysis of PBMCs, patients expressing IFN signatures and those expressing lymphoid signatures are reportedly classified in distinct clusters [48]. Therefore, our stratification method may be helpful in the appropriate selection of anti-SLE biologics because it allows direct assessment of the targeted activity of therapeutic agents.

We previously reported that SLE patients with high serum IFN-I-bioactivity have lymphopenia, low complement, high anti-dsDNA antibody titres, and high disease activity in SLE [49]. Our study also confirmed similar serological features and the presence of skin rash in patients with high serum IFN-I activity. Patients with high cutaneous lupus erythematosus disease area and severity index (CLASI) respond well to anifrolumab in the clinical trial [29]. In this context, SLE patients with blood- and skin-related symptoms might be candidates for anifrolumab. In contrast, the presence of LN correlated with high BAFF activity in sera and urine. In recent years, belimumab has been shown to increase response rates and decrease relapse rates when added to standard induction therapy for patients with active LN [21]. This increase in BAFF activity in patients with LN may explain the efficacy of belimumab in treating LN. Consequently, SLE patients with LN might be candidates for belimumab.

Limitations of this study include the small sample size as it is a single-centre study; clinical bias in patient enrolment; fewer patients with acute LN in the study of urinary BAFF measurement; influence of therapeutic agents; and inability to assess belimumab response in patients with high BAFF activity. Additionally, the function of local BAFF produced in the kidneys in patients with LN and association of these cytokine bioactivities with chronic organ damage has not yet been elucidated. Therefore, caution must be exercised to avoid overinterpreting the conclusions. Thus, further investigation is warranted to elucidate whether urinary BAFF levels reflect LN histology, the mechanism by which BAFF produced by glomerular macrophages and mesangial cells promotes renal damage, whether continuous elevation of these cytokines causes chronic organ damage, and the efficacy of belimumab in improving clinical outcomes of patients with SLE and high BAFF activity.

In conclusion, monitoring serum and urinary BAFF bioactivity with a cell-based reporter system may help identify patients with LN, and monitoring serum BAFF and IFN-I bioactivity may allow SLE stratification.

Supplementary data

Supplementary data are available at *Rheumatology* online.

Data availability statement

The data underlying this article are available in the article and in its online supplementary material.

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