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B cell-activating factors in autoimmune pulmonary alveolar proteinosis

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Abstract

Background: Autoimmune pulmonary alveolar proteinosis (APAP) results from the suppression of granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling by a neutralizing autoantibody against GM-CSF. B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are involved in immunoglobulin G production and are overproduced in various autoimmune disorders. We hypothesized that BAFF and/or APRIL levels would be elevated in serum and bronchoalveolar lavage fluid (BALF) and serum and BALF levels of BAFF and APRIL respond to the treatments (whole lung lavage (WLL) or inhalation of recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF)) in patients with APAP.

Subjects and methods: BAFF and APRIL levels in serum and BALF from 110 patients with APAP were measured at baseline and during and after treatment, using an enzyme-linked immunosorbent assay kit. We enrolled 34 healthy volunteers as serum cytokine controls, and 13 disease controls for BALF. Associations of BAFF and APRIL levels with clinical measures were assessed to clarify their clinical roles.

Results: In patients with APAP, serum BAFF and APRIL levels were significantly increased relative to healthy volunteers ($p < 0.0001$ and $p < 0.05$, respectively), and BALF BAFF and APRIL levels were significantly increased versus disease controls ($p < 0.0001$ and $p < 0.01$, respectively). Serum BAFF levels (but not APRIL levels) were significantly correlated with Krebs von den Lungen-6 (KL-6), surfactant protein (SP)-D, SP-A, and lactate dehydrogenase ($p < 0.0001$). There was no significant correlation between serum BAFF or APRIL levels and anti-GM-CSF autoantibody. BAFF and APRIL were negatively correlated with single-breath diffusion capacity for carbon monoxide (DLco) ($p = 0.004$) and forced vital capacity ($p = 0.04$), respectively. BAFF (but not APRIL) in BALF was negatively correlated with vital capacity ($p = 0.04$) and DLco ($p = 0.006$). There were significant correlations between disease severity and BAFF levels in serum ($p = 0.04$) and BALF ($p = 0.007$). Serum levels of anti-GM-CSF autoantibody, BAFF, and APRIL were not significantly affected by WLL or inhalation of recombinant human GM-CSF.

Conclusions: BAFF and APRIL levels of sera and BALF in APAP were significantly increased compared with healthy volunteer and disease control, and the BAFF and APRIL pathway might have important specific roles in pathogenesis of APAP. Our data suggest a new perspective of future treatment for APAP.

Keywords: Autoimmune pulmonary alveolar proteinosis, B cell-activating factor, Biomarker

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Introduction

Pulmonary alveolar proteinosis (PAP) is a rare lung disease with the low incidence and prevalence [1, 2]. Dysfunction of alveolar macrophages induced by a



neutralizing autoantibody against granulocyte-macrophage colony-stimulating factor (GM-CSF) is a pathogenic factor of autoimmune PAP (APAP) [2–5]. However, no correlation has been observed between the disease severity score (DSS) and levels of anti-GM-CSF autoantibody in the serum [1]. The trigger and mechanism of the overproduction of anti-GM-CSF autoantibody are not currently known.

Whole lung lavage (WLL) is the gold standard therapy for APAP [6–8]. In addition, treatment with inhaled recombinant human GM-CSF (rhGM-CSF) has been conducted in clinical trials [9–11]. The recurrence rate of APAP after WLL is unclear, although one report found that 70% of patients followed up for more than three years remained free of recurrent PAP manifestations [6]. However, another study reported that 71% of patients receiving treatment with rhGM-CSF had received WLL previously [7]. The recurrence rate of APAP after treatment with rhGM-CSF is also unclear, although it has been reported that symptoms recurred in five out of 12 patients after a mean of 6.3 months [8]. Another study reported that, during 30 months of observation following GM-CSF inhalation therapy, 12 of 35 patients required additional treatment [12]. Therefore, the development of treatments that are more effective and less invasive is desirable.

B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) play crucial roles in the selection, activation, maturation, and survival of B cells. Furthermore, these cytokines are expressed in monocytes/macrophages, dendritic cells, and activated T lymphocytes [13]. Serum levels of BAFF and APRIL increase in some autoimmune diseases, such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), rheumatoid arthritis, immunoglobulin G4 (IgG4)-related disease, and anti-Jo-1-positive polymyositis and dermatomyositis [14–18]. In SjS, overproduction of BAFF in the sputum has also been reported [19]. One report found that treatment of SLE with BAFF/APRIL inhibitor significantly improved the SLE responder index and safely decreased flares of severe disease relative to placebo plus standard SLE therapy [20]. In SLE model mice, serum levels of BAFF increased as a result of the disease, and BAFF blockade led to reduced disease manifestations [21]. Moreover, silencing of the BAFF gene in mice with collagen-induced arthritis abrogated the development of autoimmune arthritis [22].

Serum levels of BAFF and APRIL increase in autoimmune diseases in general [14–18], and levels of BAFF in the sputum increase in patients with SjS [19]. Furthermore, serum levels of BAFF are negatively correlated with pulmonary function test results [15]. However, there are no reports on B cell-activating factors in

patients with APAP, which involves overproduction of the autoantibody against GM-CSF.

We hypothesized that BAFF and/or APRIL levels in the serum and/or bronchoalveolar lavage fluid (BALF) are elevated in this disease. Therefore, we measured the levels of both BAFF and APRIL in patients with APAP, and analyzed their association with clinical measures and the effects of treatments, in the hope of identifying novel targets for treatment.

Materials and methods

Study subjects

We enrolled 115 patients with APAP (mean age: 55.8 ± 13.4 years), 34 healthy volunteers (mean age: 51.4 ± 6.5 years) as controls for serum BAFF and APRIL, and 13 non-autoimmune-disease controls (mean age: 49.2 years; range: 29.7–55.5 years; median: 49.2 years) as controls for BAFF and APRIL in BALF (Table 1). The disease controls were cases with chronic obstructive pulmonary disease ($n=4$), idiopathic pulmonary fibrosis ($n=2$), Langerhans cell histiocytosis ($n=2$), chronic bronchitis ($n=1$), Castleman disease ($n=1$), squamous cell carcinoma ($n=1$), sporadic lymphangioleiomyomatosis ($n=1$), or lymphomatoid granulomatosis ($n=1$). The diagnosis of control diseases was determined via clinical, radiological, and pathological examinations. The diagnosis of APAP was confirmed based on transbronchial lung biopsy, bronchoalveolar lavage, radiological findings, clinical findings, and the presence of anti-GM-CSF autoantibody, according to the diagnostic criteria for APAP [1, 2]. The APAP DSS was evaluated, based on symptoms and PaO_2 , and rated from least (DSS 1) to most (DSS 5) severe [1].

This study was approved by the ethics committee of the National Hospital Organization Kinki-Chuo Chest Medical Center (Sakai, Japan) (approval numbers: 365 and 576). Written informed consent was provided by all subjects.

Measurement of BAFF and APRIL in the serum and BALF

All serum samples were collected and stored at -80°C until examination.

The levels of BAFF in the serum and BALF were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the instructions provided by the manufacturer (R&D Systems, Minneapolis, MN, USA). The levels of APRIL in the serum and BALF were measured with an ELISA kit according to the instructions provided by the manufacturer (eBioscience, Vienna, Austria). These assays were performed in duplicate.

Table 1 Demographic and clinical characteristics of the subjects

Characteristics	APAP			Healthy Volunteer			Disease Control		
	N	%	Median (I.Q. range)	N	%	Median (I.Q. range)	N	%	Median (I.Q. range)
			or Mean \pm SD			or Mean \pm SD			or Mean \pm SD
Age – years	110		55.8 \pm 13.4	34		51.4 \pm 6.5	13		49.2 (29.7–55.5)
Gender									
Female	33	30		21	61.8		4	30.8	
Male	77	70		13	38.2		9	69.2	
Onset of symptom – years	110		53.0 \pm 13.3						
Symptoms									
Dyspnea	82	74.5							
Cough	54	49.1							
Sputum	39	35.5							
Dust exposure									
Yes	45	40.9							
No	65	59.1							
Smoking									
Current	28	25.5		4	11.8		3	23.1	
Ex	43	39		12	35.3		5	38.5	
Never	39	35.5		16	47.1		2	15.4	
Unknown	0	0		2	5.9		3	23.1	
Pulmonary function									
VC, % predicted	98		90.3 \pm 22.9				13		91.3 (79.8–111.0)
FVC, % predicted	98		88.9 \pm 24.0				13		91.3 (79.7–109.1)
FEV _{1.0} , % predicted	94		95.3 \pm 25.0				13		79.0 (64.1–94.6)
DLco, % predicted	97		59.1 (45.6–75.9)				13		49.4 (37.6–80.3)
PaO ₂	75		68.8 \pm 15.3						
Serum biomarkers									
KL-6, U/L	108		3799.5 (1601.0–8787.5)				13		328.0 (237.5–917.0)
SP-D, ng/ml	106		183.9 (120.6–311.5)				12		65.5 (39.0–134.8)
SP-A, ng/ml	81		96.7 (62.5–151.4)				13		40.1 (31.3–67.5)
LDH, IU/L	108		263.5 (217.8–339.8)						185.0 (144.0–219.5)
Anti-GM-CSF autoantibody, μ g/ml	110		40.5 (17.1–71.8)						

APAP, autoimmune pulmonary alveolar proteinosis; IQR, interquartile range; SD, standard deviation; VC, vital capacity; FVC, forced expiratory volume; FEV₁, forced expiratory volume in 1 s; DLco, diffusion capacity for carbon monoxide; PaO₂, arterial partial pressure of oxygen; KL-6, Krebs von den Lungen-6; SP-D, surfactant protein-D; SP-A, surfactant protein-A; LDH, lactate dehydrogenase; GM-CSF, granulocyte-macrophage colony-stimulating factor. Data are expressed as the median (IQR) or mean \pm SD

Serum biomarkers

The levels of Krebs von den Lungen-6 (KL-6) (Picolumi KL-6; EIDIA, Tokyo, Japan), SP-D (SP-D kit YAMASA EIA II; Kyowa Medex, Chiba, Japan), SP-A (SP-A test Kokusai-F kit; SYSMEX, Kobe, Japan), and lactate dehydrogenase (LDH) (Cica Liquid LDH J; Kanto Chemical, Tokyo, Japan) in the serum were measured. The reference levels of normal human serum in this study were KL-6 (< 500 U/mL), SP-D (< 110 ng/mL), SP-A (< 48.3 ng/mL), and LDH (115 < LDH < 229 IU/L).

We measured the levels of anti-GM-CSF autoantibody in the serum using a specific previously reported ELISA method [1, 23, 24] with partial modification.

Pulmonary function testing

Certified pulmonary function technicians performed pulmonary function tests using a CHESTAC-8800 (Chest M.I., Tokyo, Japan). The data collected included the percentages of vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), and single-breath diffusion capacity for carbon monoxide (DLco). Each pulmonary function measurement was expressed as a percent predicted value. Acceptability and reproducibility criteria from the American Thoracic Society's recommendations for standardization were used to assess the validity of each testing session [25].

WLL treatment

After induction of general anesthesia, patients were intubated, while supine, with a left-side double-lumen endotracheal tube and their lungs were ventilated mechanically with 100% oxygen. The lung was lavaged with warmed (37°C) normal saline using aliquots of 500 mL at a pressure of 30 cmH₂O. The lavage was terminated when the color of the lavage fluid changed from milky to clear; the total volume of saline delivered to a single lung was typically 15–20 L [26]. Recovered WLL fluid was filtered with a gauze filter and centrifuged at 44 × g for 5 min, and the supernatant was stored at –30°C until use.

Bronchoalveolar lavage

Bronchoalveolar lavage was performed as previously described [27] via flexible bronchoscopy. Aliquots of sterile saline (50 mL) at 37°C were injected thrice through the bronchoscope for clinical usage and gently aspirated with a syringe 4th BALF for this study. The BALF was filtered using a gauze filter and centrifuged at 44 × g for 5 min, and the supernatant was stored at –30°C until use.

Treatment with inhalation of recombinant human GM-CSF

Two patients received rhGM-CSF (125 µg in 2 mL of sterile saline; Molgramostim, Leucomax; Novartis AG, Basel, Switzerland) via inhalation of aerosol using an LC-PLUS nebulizer with a manual interrupter valve connected to a PARI Turbo BOY compressor (PARI, Starnberg, Germany). The treatment was administered twice daily, every second week, for 24 weeks [28]. Fifteen patients received rhGM-CSF (125 µg in 2 mL of sterile saline; Sargramostim, Leukine lyophilized formulation; Berlex, Seattle, WA, USA) via inhalation of aerosol using an LC-PLUS nebulizer with a manual interrupter valve connected to a PARI Turbo BOY compressor [10]. Treatments included the two effective regimens of varying rhGM-CSF dosage [9, 28]. Clinical response was defined as a reduction in alveolar–arterial O₂ tension difference of at least 10 torr at the end of treatment relative to baseline [9].

Statistical analysis

Statistical analyses were performed using the JMP software, Version 10.0 (SAS Institute, Cary, NC, USA). Numerical variables are expressed as median (interquartile range) and were assessed using the Wilcoxon rank-sum test. Correlations of variables were assessed using Spearman's rank correlation coefficient. Steel's test was used to compare the levels of BAFF and APRIL before rhGM-CSF inhalation and WLL with after treatment.

Differences were considered statistically significant at $p < 0.05$.

Results

Baseline levels of BAFF and APRIL in serum and BALF

The levels of BAFF and APRIL in the serum of patients with APAP were significantly higher than those in healthy volunteers ($p < 0.0001$ and $p < 0.05$, respectively; Fig. 1a, b). Median levels of BAFF in the serum were 904.8 (780.5–1,252.8) pg/mL in patients with APAP and 735.5 (652.2–891.0) pg/mL in healthy volunteers. Median levels of APRIL in the serum were 435.4 (243.3–586.5) pg/mL in patients with APAP and 303.4 (203.1–401.9) pg/mL in healthy volunteers.

The levels of BAFF and APRIL in the BALF of patients with APAP were significantly higher than those in disease controls ($p < 0.0001$ and $p < 0.01$, respectively; Fig. 1c, d). Median levels of BAFF in the BALF were 191.4 (88.4–703.3) pg/mL in patients with APAP and 8.1 (4.85–26.9) pg/mL in disease controls. Median levels of APRIL in the BALF were 639.5 (237.5–1,026.8) pg/mL in patients with APAP and 201.0 (118.5–303.0) pg/mL in disease controls. There were two cases that showed a marked increase in serum levels of BAFF (Fig. 1). Of these, one had BAFF levels of 6737.5 pg/mL and was complicated by non-tuberculous mycobacteriosis, and serum anti GM-CSF autoantibody level was 36.9 µg/mL. The other had the BAFF level of 4910.4 pg/mL, exhibited a marked increase I KL-6 (70,010 U/mL), carcinoembryonic antigen (80.0 ng/mL), cytokeratin subunit 19 fragment (53.0 ng/mL), and was complicated by myelodysplastic syndrome (MDS). Serum anti-GM-CSF autoantibody was 21.4 µg/mL. Pathological and cytological findings of PAP were confirmed about these two patients, and our final diagnosis were APAP (not secondary PAP) complicated with NTM or MDS from the definition of APAP.

Levels of both BAFF and APRIL in the serum and BALF were correlated ($p = 0.009$ and $p = 0.001$, respectively). However, levels of BAFF in the serum and APRIL in the BALF were negatively correlated ($p = 0.005$; Table 2).

The baseline levels of BAFF and APRIL in the serum and BALF were not correlated with dyspnea, cough, sputum, smoking, or exposure to dust (Table 3). The levels of BAFF in the serum and BALF were significantly correlated with DSS ($p = 0.04$ and $p = 0.007$, respectively). However, levels of APRIL in the serum and BALF were not correlated with DSS (Table 3).

Correlations between BAFF, APRIL, and serum biomarkers at baseline

We confirmed the previously reported elevation of serum biomarkers for APAP in patients with APAP [1,

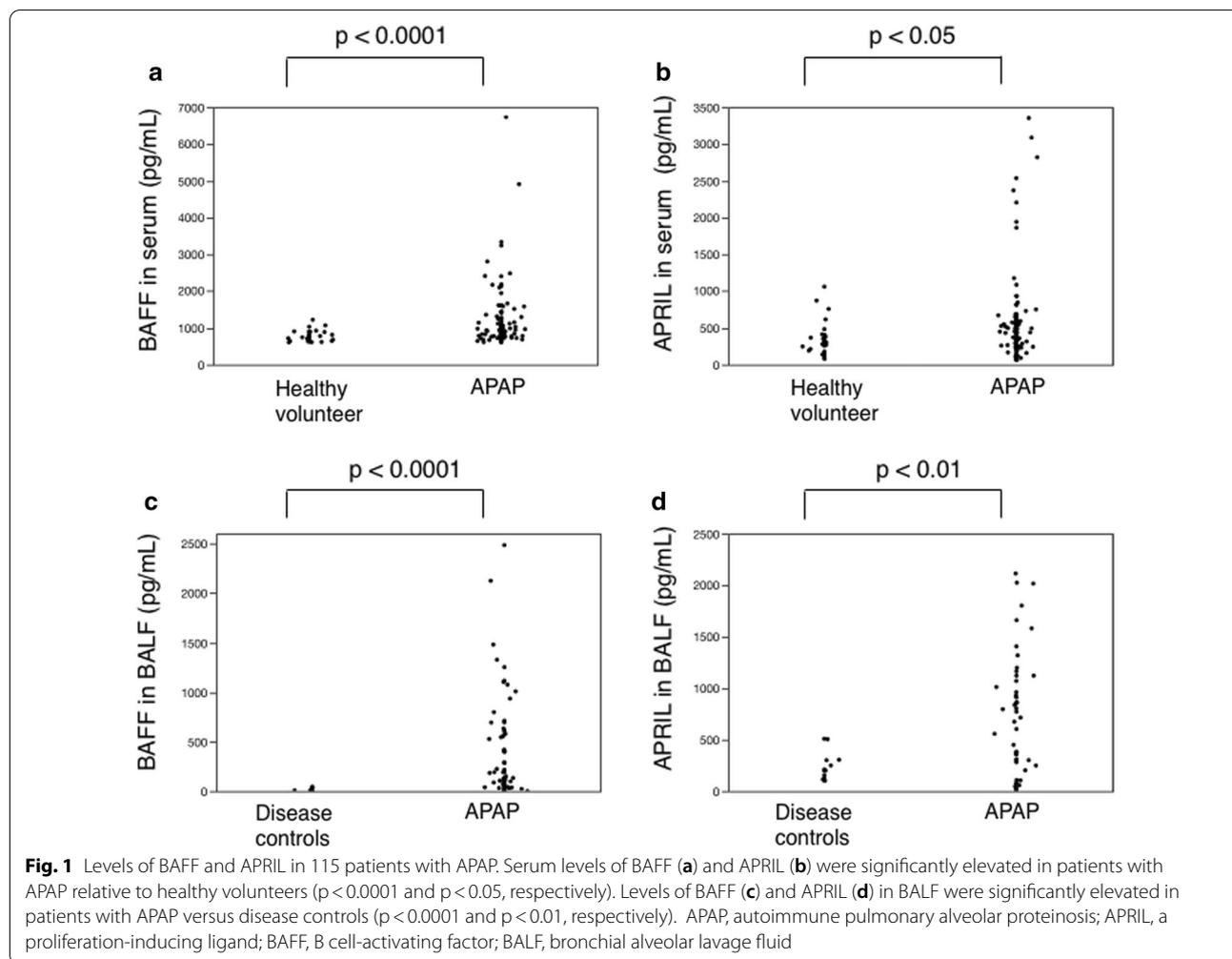


Table 2 Association between B cell-activating factors in the serum and BALF

	BAFF in serum	APRIL in serum	BAFF in BALF	APRIL in BALF
BAFF in serum		0.14 (0.14)	0.33 (0.009*)	- 0.40 (0.004*)
APRIL in serum			0.09 (0.51)	0.36 (0.001*)
BAFF in BALF				- 0.27 (0.06)
APRIL in BALF				

BAFF, B cell-activating factor; APRIL, a proliferation-inducing ligand; BALF, bronchoalveolar lavage fluid. Data were analyzed using Spearman’s correlation coefficient. *Significant at the 0.05 level

28, 29]. The levels of KL-6, SP-D, SP-A, and LDH in the serum were significantly correlated with those of BAFF ($p < 0.0001$). However, only the levels of APRIL in the serum were correlated with SP-D ($p = 0.04$). Serum levels of BAFF and APRIL were not correlated with anti-GM-CSF autoantibody (Table 4). Among the serum biomarkers examined in this study, only SP-A showed

correlation with anti-GM-CSF autoantibody (data not shown).

Correlations between BAFF and APRIL levels and pulmonary functions at baseline

We evaluated the relationship between the levels of BAFF and APRIL (in the serum and BALF) and pulmonary functions at baseline. Serum levels of BAFF were

Table 3 Correlations between symptoms and baseline levels of BAFF and APRIL

Symptom	BAFF		APRIL	
	Serum	BALF	Serum	BALF
Dyspnea	0.15	0.08	0.38	0.18
Cough	0.80	0.98	0.81	0.72
Sputum	0.40	0.97	0.54	0.67
Smoking	0.30	0.18	0.33	0.26
Exposure to dust	0.79	0.32	0.65	0.31
DSS	0.04*	0.007*	0.25	0.90

BAFF, B cell-activating factor; APRIL, a proliferation-inducing ligand; DSS, disease severity score. Data except DSS were analyzed using Wilcoxon rank-sum test. Data of DSS were analyzed using Spearman's correlation coefficient. *Significant at the 0.05 level

Table 4 Correlations between serum biomarkers and baseline levels of BAFF and APRIL

Biomarker	BAFF		APRIL	
	ρ	p	ρ	p
KL-6	0.50	<0.0001*	0.15	0.12
SP-D	0.48	<0.0001*	0.20	0.04*
SP-A	0.43	<0.0001*	0.15	0.17
LDH	0.47	<0.0001*	0.13	0.17
GM-CSF autoantibody	0.13	0.19	0.07	0.14

Abbreviations: BAFF, B cell-activating factor; APRIL, a proliferation-inducing ligand; KL-6, Krebs von den Lungen-6; SP-D, surfactant protein-D; SP-A, surfactant protein-A; LDH; lactate dehydrogenase; GM-CSF, granulocyte-macrophage colony-stimulating factor. Data were analyzed using Spearman's correlation coefficient. *Significant at the 0.05 level

Table 5 Correlations between pulmonary functions and baseline levels of BAFF and APRIL in serum

Pulmonary function	BAFF in serum		APRIL in serum	
	ρ	p	ρ	p
VC (% predicted)	-0.17	0.10	-0.15	0.15
FVC (% predicted)	-0.14	0.17	-0.21	0.04*
FEV1 (% predicted)	-0.08	0.46	-0.04	0.71
DLco (% predicted)	-0.29	0.004*	-0.15	0.15

BAFF, B cell-activating factor; APRIL, a proliferation-inducing ligand; VC, vital capacity; FVC, forced expiratory volume; FEV1, forced expiratory volume in 1 s; DLco, diffusion capacity for carbon monoxide. Data were analyzed using Spearman's correlation coefficient. *Significant at the 0.05 level

significantly negatively correlated with % predicted DLco (p=0.004), but were not correlated with % predicted VC, FVC, or FEV1 (Table 5). Serum levels of APRIL were significantly negatively correlated with % predicted FVC (p=0.04), but were not significantly correlated with % predicted VC, FEV1, or DLco (Table 5).

Table 6 Correlations between pulmonary functions and levels of BAFF and APRIL in BALF

Pulmonary function	BAFF in BALF		APRIL in BALF	
	ρ	p	ρ	p
VC (% predicted)	-0.29	0.04*	-0.22	0.14
FVC (% predicted)	-0.24	0.07	-0.25	0.10
FEV1 (% predicted)	-0.08	0.59	-0.002	0.99
DLco (% predicted)	-0.34	0.006*	-0.05	0.77

BAFF, B cell-activating factor; APRIL, a proliferation-inducing ligand; BALF, bronchoalveolar lavage fluid; VC, vital capacity; FVC, forced expiratory volume; FEV1, forced expiratory volume in 1 s; DLco, diffusion capacity for carbon monoxide. Data were analyzed using Spearman's correlation coefficient. *Significant at the 0.05 level

The levels of BAFF in the BALF were significantly negatively correlated with % predicted VC (p=0.04) and DLco (p=0.006), but there were no significant correlations between APRIL in the BALF and the pulmonary functions (Table 6).

Effects of treatment on serum levels of BAFF and APRIL

Seventeen patients with APAP were treated with rhGM-CSF. We evaluated their serum BAFF and APRIL levels at baseline and after 12 and 24 weeks of treatment, but did not detect any significant treatment effects (Fig. 2a and c) same as anti-GM-CSF autoantibody (data not shown). However, we confirmed the improvement of serum level of KL-6 and DSS with rhGM-CSF (data not shown) as in the previous report [12, 28]. In order to assess the effects of rhGM-CSF inhalation on BAFF and APRIL, the patients were treated with inhaled GM-CSF under the protocols previously reported [9, 28]. In fact, serum levels of BAFF and APRIL were unaffected regardless of response to rhGM-CSF inhalation therapy and despite differences in history of smoking and dust exposure (data not shown).

WLL was performed in 16 patients with APAP, and we evaluated their serum levels of BAFF and APRIL at baseline and two and seven days after treatment. Similarly to our findings for GM-CSF inhalation therapy, we did not detect a significant effect of the treatment on the serum levels of BAFF or APRIL (Fig. 2b, d).

Discussion

This is the first report to demonstrate the overproduction of the B cell-activating factors BAFF and APRIL in patients with APAP relative to healthy volunteers (in the serum) and disease controls (in the BALF) (Fig. 1). Furthermore, the levels of BAFF in both serum and BALF were correlated with DSS (Table 3), and the serum levels were correlated with serum biomarkers for APAP (Table 4). However, the treatments for APAP (i.e.,

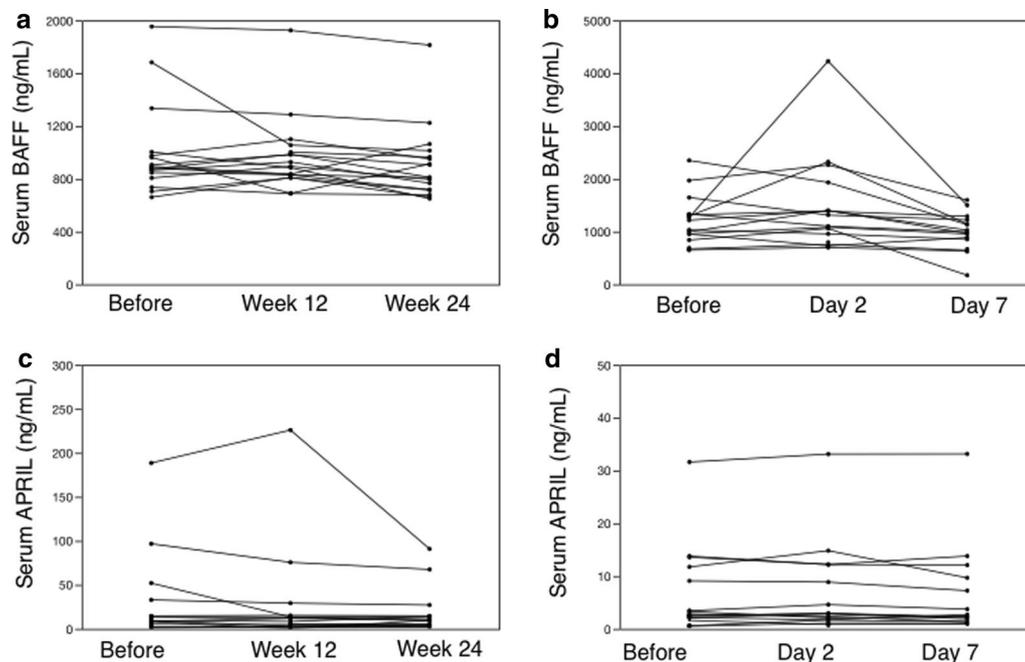


Fig. 2 Change in serum levels of BAFF and APRIL before and after treatment with **a** rhGM-CSF inhalation therapy (N = 17) and **b** whole lung lavage (N = 16). Change in serum levels of APRIL before and after treatment with **c** rhGM-CSF inhalation (N = 17) and **d** whole lung lavage (N = 16). Abbreviations: BAFF, B cell-activating factor; APRIL, a proliferation-inducing ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor. Data were analyzed using the Steel's test

inhalation of rhGM-CSF and WLL) did not affect serum levels of BAFF or APRIL (Fig. 2), or those of the anti-GM-CSF autoantibody.

Baseline levels of BAFF and APRIL in the serum and BALF

As predicted by our hypothesis, serum levels of BAFF and APRIL were elevated in patients with APAP, as in other autoimmune diseases [14, 16–18]. However, median serum levels of BAFF in patients with APAP were approximately 32%, 40%, and 15% of those reported in SLE [30], IgG4-related disease [14], and SjS [31], respectively, and those of APRIL were approximately 5%, 20%, and 20% of those in SLE [30], IgG4-related disease [14], and SjS [32], respectively. This suggests that autoimmunity such as production of autoantibody in APAP may be lower than in these three other autoimmune diseases.

Two cases showed a marked increase in serum BAFF levels as indicated above (Fig. 1). In one of these, the patient's condition was complicated by non-tuberculous mycobacteriosis. One study has shown that the dramatic elevation of BAFF and APRIL levels in the plasma and pleural effusion of patients with tuberculous pleurisy, and the BAFF/APRIL system itself, was closely related to the T helper 1 (Th1) immune response [33]. The enhancement of BAFF and APRIL

in APAP, an organ-specific autoimmune disease, suggests an association between its pathogenesis and the Th1 immune response. In the other case, the case was complicated by MDS. The pathogenic mechanism of MDS might affect serum BAFF level via functional and qualitative abnormalities of T cell, B cell, and Natural killer cell, but the clear mechanism remains unclear [34, 35].

Although clinical symptoms were not correlated with BAFF and APRIL in the serum or BALF, BAFF levels in both were correlated with DSS (Table 3). This suggests that BAFF (rather than APRIL) may be important for disease progression. In the circulation system, BAFF occurs in soluble trimmer form and APRIL is present in both soluble trimmer form and as a multimer form in association with heparin sulfate proteoglycans. In addition, there are three types of receptors for BAFF and APRIL, which exhibit different binding abilities [13]. Measurement of BAFF may facilitate non-invasive determination of DSS without arterial blood sampling. The correlation of the levels of BAFF in the serum and BALF (Table 2) were similar to results previously reported for patients with sarcoidosis [36]. These results suggest that BAFF may be more closely associated than APRIL with the pathogenesis of APAP.

Correlations between BAFF, APRIL, and serum biomarkers at baseline

We confirmed the high concentrations of serum biomarkers that are useful for APAP diagnosis and disease evaluation [1, 28, 29]. In addition, these serum biomarkers were correlated with serum levels of BAFF, but not APRIL (Table 4). Based on experimental data regarding B cell depletion, both of these cytokines release signals to promote the differentiation and longevity of B cells, although certain immune-modulating aspects may differ [30]. The different binding abilities of the receptors for BAFF and APRIL [13] may affect the etiology and progression of APAP.

We confirmed that the levels of anti-GM-CSF autoantibody did not reflect the DSS, as previously reported for APAP [37, 38]. However, we did find that BAFF was correlated with the DSS (Table 4). Since BAFF is a B cell-activation factor, this suggests that B cell activation affects the pathogenesis of APAP. BAFF exhibits similar behavior to that of the serum biomarkers for APAP, and may thus be a promising biomarker for disease progression.

Correlations between BAFF and APRIL levels and pulmonary functions at baseline

The baseline levels of BAFF in the serum and BALF were negatively correlated with baseline DLco (Tables 5, 6). The concentration of BAFF is high in cases that are so severe that gas exchange problems occur, so the correlation between BAFF levels and DSS makes sense (Table 3). The age of the APAP patients participating in this study may also have exacerbated their disease severity, since it is an extrapulmonary factor connected with decreasing diffusion capacity.

Effects of treatment on serum levels of BAFF and APRIL

In our study, serum levels of BAFF and APRIL did not change significantly after WLL or rhGM-CSF inhalation therapy (Fig. 2). Although BAFF and APRIL present locally in the lungs are temporarily removed by WLL, they are soon replenished because the treatment does not remove the cells that produce them [39]. In contrast, it is conceivable that rhGM-CSF inhalation therapy exerts a negative effect on BAFF and APRIL levels, because the alveolar macrophages activated by rhGM-CSF treatment are one of the types of cells that produce BAFF and APRIL. The reason for the ineffectiveness of the current APAP treatments in reducing BAFF and APRIL levels may be that these treatments do not act directly on the concentrations of these cytokines. Interestingly, there were some cases with decreased levels of BAFF and APRIL after treatment. However, these differences were not dependent on a history of smoking [1] or dust

exposure [40], which are considered to be risk factors for APAP (data not shown). There may also be other reasons why the treatments did not directly affect the levels of BAFF, APRIL, or the anti-GM-CSF autoantibody in APAP. Neutrophils producing BAFF maintain autoantibody production in settings of autoimmunity and cancer [41]. Hence, it has been suggested that the use of medicine that suppresses B cell-activating factors, such as belimumab [42], may be effective for the treatment of APAP. There were reports about the effectiveness of anti-CD20 antibody (rituximab) treatment on pulmonary functions [43] and on alveolar macrophage lipid metabolism by increasing lipid transport and surface catabolism in autoimmune pulmonary alveolar proteinosis. Suggested mechanisms of these effects are GM-CSF stimulation of alveolar macrophage ATP-binding cassette transporter lipid homeostasis and Lysosomal phospholipase A2 [44]. CD20 is widely expressed from naïve B cell to memory B cell. However, rituximab does not affect plasma cells which lack CD20 expression. Since the BAFF receptor is also expressed in plasma cells [45, 46], a therapy targeting BAFF could be effective on plasma cells too. In our study, inhaled rhGM-CSF and WLL could not attenuate serum levels of BAFF and APRIL in APAP. There are several reports that serum BAFF levels increased after the treatment of anti-CD20 antibody [45] in patients with autoimmune diseases.

Suppression of B cell-activating factors could be a new candidate of future APAP treatment as suggested in autoimmune diseases [45]. Further multicenter longitudinal studies are warranted to further investigate the role of BAFF and APRIL and therapeutic target in APAP.

Limitations

There were several limitations in this study, including the retrospective nature of the investigation, the single-center cohort, and the small number of patients with non-autoimmune diseases.

Conclusions

BAFF and APRIL levels of sera and BALF in APAP were significantly increased compared with healthy volunteer and disease control, and the BAFF and APRIL pathway might have important specific roles in pathogenesis of APAP. Our data suggest a new perspective of future treatment for APAP.

Abbreviations

APAP: Autoimmune pulmonary alveolar proteinosis; GM-CSF: Granulocyte-macrophage colony-stimulating factor; BAFF: B cell-activating factor; APRIL: A proliferation-inducing ligand; WLL: Whole lung lavage; BALF: Bronchoalveolar lavage fluid; DSS: Disease severity score; SLE: Systemic lupus erythematosus; SJS: Sjögren's syndrome; KL-6: Krebs von den Lungen-6; SP: Surfactant protein; LDH: Lactate dehydrogenase; ELISA: Enzyme-linked immunosorbent assay;

VC: Vital capacity; FVC: Forced vital capacity; FEV1: Forced expiratory volume in one second; DLco: Single-breath diffusion capacity for carbon monoxide;; Th: T helper.

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Authors' contributions

Conceptualization: MH, YI. Data curation: MH, TA, CS, TT, RS, SM, SS, NT, KK, YI, TK. Pathological evaluation: TK. Radiological evaluation: MA. Formal analysis: MH, YI. Funding acquisition: MH, TA, MA, YI. Investigation: MH, YI. Methodology: MH, YI. Project administration: YI. Supervision: YI. Writing of original draft: MH, YI. Writing, review and editing: MH, TA, YI. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available, to ensure the anonymity of the subjects. The datasets are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

We obtained written informed consent from all subjects prior to prospective assignment to the cohort, data collection, and serum collection (approval number: 365). The present study was also approved by the institutional review board of the Kinki-Chuo Chest Medical Centre, Sakai City, Osaka, Japan (approval number: 576).

Consent for publication

All subjects of this study provided written informed consent for the publication of the present findings.

Competing interests

YI is a member of the advisory board of SAVARA and steering committee of Boehringer Ingelheim regarding in this study. There are no other conflicts of interest to report.

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References

- Inoue Y, Trapnell BC, Tazawa R, Arai T, Takada T, Hizawa N, et al. Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. *Am J Respir Crit Care Med*. 2008;177(7):752–62.
- Kumar A, Abdelmalak B, Inoue Y, Culver DA. Pulmonary alveolar proteinosis in adults: pathophysiology and clinical approach. *Lancet Respir Med*. 2018;6(7):554–65.
- Kitamura T, Tanaka N, Watanabe J, Uchida S, Kanegasaki, Yamada Y, et al. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med*. 1999;190(6):875–80.
- Sakagami T, Uchida K, Suzuki T, Carey BC, Wood RE, Wert SE, et al. Human GM-CSF autoantibodies and reproduction of pulmonary alveolar proteinosis. *N Engl J Med*. 2009;361(27):2679–81.
- Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med*. 2003;349(26):2527–39.
- Beccaria M, Luisetti M, Rodi G, Corsico A, Zoia MC, Colato S, et al. Long-term durable benefit after whole lung lavage in pulmonary alveolar proteinosis. *Eur Respir J*. 2004;23(4):526–31.
- Seymour JF, Presneil JJ, Schoch OD, Downie GH, Moore PE, Doyle IR, et al. Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. *Am J Respir Crit Care Med*. 2001;163(2):524–31.
- Wylam ME, Ten R, Prakash UB, Nadrous HF, Clawson ML, Anderson PM. Aerosol granulocyte-macrophage colony-stimulating factor for pulmonary alveolar proteinosis. *Eur Respir J*. 2006;27(3):585–93.
- Tazawa R, Trapnell BC, Inoue Y, Arai T, Takada T, Nasuhara Y, et al. Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med*. 2010;181(12):1345–54.
- Tazawa R, Ueda T, Abe M, Tatsumi K, Eda R, Kondoh S, et al. Inhaled GM-CSF for pulmonary alveolar proteinosis. *N Engl J Med*. 2019;381(10):923–32.
- Trapnell BC, Inoue Y, Bonella F, Morgan C, Jouneau S, Bendstrup E, et al. Inhaled molgramostim therapy in autoimmune pulmonary alveolar proteinosis. *New Engl J Med*. 2020.
- Tazawa R, Inoue Y, Arai T, Takada T, Kasahara Y, Hojo M, et al. Duration of benefit in patients with autoimmune pulmonary alveolar proteinosis after inhaled granulocyte-macrophage colony-stimulating factor therapy. *Chest*. 2014;145(4):729–37.
- Vincent FB, Saulep-Easton D, Figgett WA, Fairfax KA, Mackay F. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. *Cytokine Growth Factor Rev*. 2013;24(3):203–15.
- Kiyama K, Kawabata D, Hosono Y, Kitagori K, Yukawa N, Yoshifuji H, et al. Serum BAFF and APRIL levels in patients with IgG4-related disease and their clinical significance. *Arthritis Res Ther*. 2012;14(2):R86.
- Krystufkova O, Hulejova H, Mann HF, Pecha O, Putova I, Ekholm L, et al. Serum levels of B-cell activating factor of the TNF family (BAFF) correlate with anti-Jo-1 autoantibodies levels and disease activity in patients with anti-Jo-1 positive polymyositis and dermatomyositis. *Arthritis Res Ther*. 2018;20(1):158.
- Mariette X. [Pathophysiology of Sjogren's syndrome]. *Ann Med Interne (Paris)*. 2003;154(3):157–68.
- Scapini P, Carletto A, Nardelli B, Calzetti F, Roschke V, Merigo F, et al. Pro-inflammatory mediators elicit secretion of the intracellular B-lymphocyte stimulator pool (BLyS) that is stored in activated neutrophils: implications for inflammatory diseases. *Blood*. 2005;105(2):830–7.
- Vincent FB, Northcott M, Hoi A, Mackay F, Morand EF. Clinical associations of serum interleukin-17 in systemic lupus erythematosus. *Arthritis Res Ther*. 2013;15(4):R97.
- Nilsson AM, Tufvesson E, Hesselstrand R, Olsson P, Wollmer P, Mandl T. Increased B-cell activating factor, interleukin-6, and interleukin-8 in induced sputum from primary Sjogren's syndrome patients. *Scand J Rheumatol*. 2019;48(2):149–56.
- Stohl W, Schwarting A, Okada M, Scheinberg M, Doria A, Hammer AE, et al. Efficacy and safety of subcutaneous belimumab in systemic lupus erythematosus: a fifty-two-week randomized, double-blind, Placebo-controlled study. *Arthritis Rheumatol*. 2017;69(5):1016–27.
- Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature*. 2000;404(6781):995–9.
- Lai Kwan Lam Q, King Hung Ko O, Zheng BJ, Lu L. Local BAFF gene silencing suppresses Th17-cell generation and ameliorates autoimmune arthritis. *Proc Natl Acad Sci U S A*. 2008;105(39):14993–8.

23. Kitamura T, Uchida K, Tanaka N, Tsuchiya T, Watanabe J, Yamada Y, et al. Serological diagnosis of idiopathic pulmonary alveolar proteinosis. *Am J Respir Crit Care Med*. 2000;162(2 Pt 1):658–62.
24. Uchida K, Nakata K, Suzuki T, Luisetti M, Watanabe M, Koch DE, et al. Granulocyte/macrophage-colony-stimulating factor autoantibodies and myeloid cell immune functions in healthy subjects. *Blood*. 2009;113(11):2547–56.
25. Enright P. Flawed interpretative strategies for lung function tests harm patients. *Eur Respir J*. 2006;27(6):1322–3. author reply 3–4.
26. Sugimoto C, Arai T, Nishiyama A, Inoue Y, Kagawa T, Akira M, et al. [Multidisciplinary assessment of effects, safety and procedure of whole lung lavage for 8 patients with autoimmune pulmonary alveolar proteinosis]. *Jpn Respir Soc*. 2011;49(8):569–76.
27. Inoue Y, Barker E, Daniloff E, Kohno N, Hiwada K, Newman LS. Pulmonary epithelial cell injury and alveolar-capillary permeability in berylliosis. *Am J Respir Crit Care Med*. 1997;156(1):109–15.
28. Tazawa R, Hamano E, Arai T, Ohta H, Ishimoto O, Uchida K, et al. Granulocyte-macrophage colony-stimulating factor and lung immunity in pulmonary alveolar proteinosis. *Am J Respir Crit Care Med*. 2005;171(10):1142–9.
29. Arai T, Inoue Y, Sugimoto C, Inoue Y, Nakao K, Takeuchi N, et al. CYFRA 21 – 1 as a disease severity marker for autoimmune pulmonary alveolar proteinosis. *Respirology*. 2014;19(2):246–52.
30. Vallerskog T, Heimbürger M, Gunnarsson I, Zhou W, Wahren-Herlenius M, Trollmo C, et al. Differential effects on BAFF and APRIL levels in rituximab-treated patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Res Ther*. 2006;8(6):R167.
31. Mariette X, Roux S, Zhang J, Bengoufa D, Lavie F, Zhou T, et al. The level of BlyS (BAFF) correlates with the titre of autoantibodies in human Sjogren's syndrome. *Ann Rheum Dis*. 2003;62(2):168–71.
32. Kontny E, Lewandowska-Poluch A, Chmielinska M, Olesinska M. Subgroups of Sjogren's syndrome patients categorised by serological profiles: clinical and immunological characteristics. *Reumatologia*. 2018;56(6):346–53.
33. Liu K, Zhang Y, Hu S, Yu Y, Yang Q, Jin D, et al. Increased levels of BAFF and APRIL related to human active pulmonary tuberculosis. *PLoS One*. 2012;7(6):e38429.
34. Economopoulos T, Economidou J, Giannopoulos G, Terzoglou C, Papa-georgiou E, Dervenoulas J, et al. Immune abnormalities in myelodysplastic syndromes. *J Clin Pathol*. 1985;38(8):908–11.
35. Enright H, Miller W. Autoimmune phenomena in patients with myelodysplastic syndromes. *Leuk Lymphoma*. 1997;24(5–6):483–9.
36. Ando M, Goto A, Takeno Y, Yamasue M, Komiya K, Umeki K, et al. Significant elevation of the levels of B-cell activating factor (BAFF) in patients with sarcoidosis. *Clin Rheumatol*. 2018;37(10):2833–8.
37. Arai T, Hamano E, Inoue Y, Ryushi T, Nukiwa T, Sakatani M, et al. Serum neutralizing capacity of GM-CSF reflects disease severity in a patient with pulmonary alveolar proteinosis successfully treated with inhaled GM-CSF. *Respir Med*. 2004;98(12):1227–30.
38. Inoue Y, Nakata K, Arai T, Tazawa R, Hamano E, Nukiwa T, et al. Epidemiological and clinical features of idiopathic pulmonary alveolar proteinosis in Japan. *Respirology*. 2006;11 Suppl:55–60.
39. Vincent FB, Morand EF, Schneider P, Mackay F. The BAFF/APRIL system in SLE pathogenesis. *Nat Rev Rheumatol*. 2014;10(6):365–73.
40. Bonella F, Bauer PC, Griese M, Ohshimo S, Guzman J, Costabel U. Pulmonary alveolar proteinosis: new insights from a single-center cohort of 70 patients. *Respir Med*. 2011;105(12):1908–16.
41. Roosnek E, Burjanadze M, Dietrich PY, Matthes T, Passweg J, Huard B. Tumors that look for their springtime in APRIL. *Crit Rev Oncol Hematol*. 2009;72(2):91–7.
42. Samy E, Wax S, Huard B, Hess H, Schneider P. Targeting BAFF and APRIL in systemic lupus erythematosus and other antibody-associated diseases. *Int Rev Immunol*. 2017;36(1):3–19.
43. Kavuru MS, Malur A, Marshall I, Barna BP, Meziane M, Huizar I, et al. An open-label trial of rituximab therapy in pulmonary alveolar proteinosis. *Eur Respir J*. 2011;38(6):1361–7.
44. Malur A, Kavuru MS, Marshall I, Barna BP, Huizar I, Karnekar R, et al. Rituximab therapy in pulmonary alveolar proteinosis improves alveolar macrophage lipid homeostasis. *Respir Res*. 2012;13:46.
45. Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. *Nat Rev Drug Discov*. 2020.
46. Stohl W. BlySfulness does not equal blissfulness in systemic lupus erythematosus: a therapeutic role for BlyS antagonists. *Curr Dir Autoimmun*. 2005;8:289–304.

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