Clinical and Experimental Immunology, 2023, **213**, 125–137
https://doi.org/10.1093/cei/uxad024
Advance access publication 16 February 2023
Research Article

Clinical & Experimental IMMUNOLOGY

OXFORD

Research Article

Features of BAFF and APRIL receptors on circulating B cells in antineutrophil cytoplasmic antibody-associated vasculitis

Yasuhiro Shimojima*, Dai Kishida, Takanori Ichikawa, Ryota Takamatsu, Shun Nomura and Yoshiki Sekijima

Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan

*Correspondence: Yasuhiro Shimojima, Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. Email: yshimoji@shinshu-u.ac.jp

Abstract

To investigate the features of circulating B cells, their expressing receptors, serum levels of B-cell activation factor of the TNF family (BAFF), and a proliferation-inducing ligand (APRIL) in antineutrophil cytoplasmic antibody-associated vasculitis (AAV). Blood samples from 24 patients with active AAV (a-AAV), 13 with inactive AAV (i-AAV), and 19 healthy controls (HC) were included in this study. The proportion of B cells and their expressing BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen were analyzed via flow cytometry. Serum levels of BAFF, APRIL, and interleukin (IL)-4, IL-6, IL-10, and IL-13 were also evaluated using an enzyme-linked immunosorbent assay. The proportion of plasmablasts (PB)/plasma cells (PC) and serum levels of BAFF, APRIL, IL-4, and IL-6 were significantly higher in a-AAV than in HC. Higher serum levels of BAFF, APRIL, and IL-4 were observed in i-AAV than in HC. Lower expression of BAFF-R on memory B cells and higher expression of TACI on CD19+ cells, immature B cells, and PB/PC were demonstrated in a-AAV and i-AAV than in HC. The population of memory B cells was positively associated with serum APRIL levels and BAFF-R expression in a-AAV. In conclusion, decreased expression of BAFF-R on memory B cells and increased expression of TACI on CD19+ cells, immature B cells, and PB/PC, as well as increased serum levels of BAFF and APRIL, were sustained even in the remission phase of AAV. Persistent aberrant signaling of BAFF/APRIL may contribute to disease relapse.

Keywords: ANCA-associated vasculitis, BAFF, APRIL, BAFF-R, TACI, B-cell subsets

Abbreviations: APRIL: a proliferation-inducing ligand; BAFF/APRIL receptors: three different types of receptors to which BAFF and APRIL bind; BAFF: B-cell activation factor of the TNF family; BAFF-R: BAFF receptor; BCMA: B-cell maturation antigen; BVAS: Birmingham Vasculitis Activity Score; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; GPA: granulomatosis with polyangiitis; HC: healthy controls; i-AAV: the inactive AAV group; IL: interleukin; MPA: microscopic polyangiitis; MPO: myeloperoxidase; PB: plasmablasts; PC: plasma cells; PR3: proteinase 3; RTX: rituximab; TACI: transmembrane activator and calcium modulator and cyclophilin ligand interactor; TNF: tumor necrosis factor; WBC: white blood cells.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic autoimmune disorder characterized by a pauci-necrotizing small- to medium-sized vasculitis as well as the participation of ANCA targeting myeloperoxidase (MPO) and proteinase 3 (PR3) [1, 2]. Subtypes of ANCA are also crucial for determining the AAV classification including microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis. Although some etiologies including various susceptibility genes and environmental inducers have been suggested as contributors to the development of AAV, innate and acquired immune systems, whose immune regulatory functions are insufficient, have been found to promote the formation of vasculitis and granuloma lesions [2–5]. B-lineage cells play crucial roles not only in promoting antigen presentation and secreting pro-inflammatory cytokines but also in producing disease-specific autoantibodies, such as autoreactive B cells that produce MPO- and PR3-ANCA that fundamentally participate in the pathogenesis of AAV [2, 3]. Moreover, it is necessary to elucidate the immunological mechanism underlying the activation of B cell lineage to thoroughly understand the pathogenesis of AAV.

B-cell activation factor of the tumor necrosis factor (TNF) family (BAFF) and a proliferation-inducing ligand (APRIL) are well-known factors that promote the survival and activation of B-cells [6–8]. BAFF and APRIL are produced by innate immune cells, including macrophages, neutrophils, monocytes, and dendritic cells [7–9]. Increased production of BAFF and APRIL have been identified in several autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), Sjögren syndrome (SJS), and multiple sclerosis [9–12]. Some studies on AAV have also demonstrated increased levels of BAFF and APRIL in the

Received 27 September 2022; Revised 11 February 2023; Accepted for publication 15 February 2023

active phase of the disease [12–15]. BAFF and APRIL induce biological activities by binding to three different types of receptors (BAFF/APRIL receptors) classified as membrane proteins of the TNF receptor superfamily; BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA) [6, 16]. BAFF/APRIL receptors are expressed on the membrane surface of immunocompetent cells, especially in B cells. BAFF has a physiological affinity for BAFF-R, TACI, and BCMA, whereas APRIL ordinarily binds to TAC and BCMA and has no affinity for BAFF-R [16, 17]. However, it is uncertain how the expression of BAFF/APRIL receptors could be altered depending on disease activity in AAV. Although some studies on BAFF/APRIL receptors have been conducted in SLE, SJS, neoplasms, or hematologic diseases to date [6, 18, 19], their features varied among diseases.

In this study, we investigated the features of circulating B cells, their BAFF/APRIL receptors, and serum levels of BAFF, APRIL, and other cytokines implicated in the differentiation of B-cell lineages in patients with AAV; in addition, we focused on their relationships.

Materials and methods

Patients and samples

A total of 24 patients (mean age, 67 years; 8 males and 16 females), who had not undergone immunosuppressive treatment, were enrolled in the active AAV group (a-AAV). Of the patients with a-AAV, MPA, and GPA were classified 13 (54%) and 11 (46%) patients, respectively. The classification of MPA or GPA was determined according to the criteria of the Chapel Hill Consensus Conference [1] and/or the consensus algorithm proposed by the European Medicines Agency [20]. The median (interquartile range [IQR]) Birmingham Vasculitis Activity Score (BVAS) [21] in this group was 14.0 (11.5–19.8). Thirteen patients (mean age, 67 years; five males and eight females), whose BVAS was zero, were also enrolled in the inactive AAV group (i-AAV). The median (IQR) disease duration in this group was 40 (24–48) months after initiating immunosuppressive therapy. All patients were on maintenance therapy that included prednisolone (median (IQR) 5 (5–7) mg daily) (n = 12), methotrexate (n = 4), azathioprine (n = 6), and mizoribine (n = 1). Of the 13 patients with i-AAV, 4 were primarily administered remission induction therapy with rituximab (RTX) and 3 were given an intravenous infusion of cyclophosphamide. In addition, 19 age-matched healthy controls (HC) (mean age, 59 years; 9 males and 10 females), were included in the control group for comparison. Whole blood samples, which were collected into EDTA-coated tubes, were obtained from 24 patients with a-AAV before initiating immunosuppressive therapy, 13 with i-AAV, and 19 HC. Of the 24 patients with a-AAV, 7 were later added to the 13 patients with i-AAV as consecutive patients after remission.

Clinical parameters

Clinical involvements based on BVAS and laboratory findings, including positivity for MPO-ANCA or PR3-ANCA, the number of white blood cells (WBC), neutrophils, lymphocytes, serum levels of C-reactive protein (CRP), and estimated glomerular filtration rate (eGFR), were compared between the patients with a-AAV and i-AAV (Supplementary Table S1). Clinical and laboratory findings were recorded when blood samples were provided.

Phenotypical analyses

Phenotypical analyses of peripheral blood B cells were performed using flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples using gradient centrifugation with Ficoll-Hypaque PLUS (GE Healthcare, Pittsburgh, PA, USA). Isolated PBMCs were stained with FITC-conjugated anti-CD19 (BioLegend, San Diego, CA, USA), PE-conjugated anti-CD38 (Beckman Coulter, Brea, CA, USA), and Pacific blue-conjugated anti-CD27 (BioLegend) antibodies, as well as with APC-conjugated anti-BAFF-R (CD268, Miltenyi Biotec, Bergisch Gladbach, Germany), APC-conjugated anti-TACI (CD267, BioLegend), or APC-conjugated anti-BCMA (CD269, Miltenyi Biotec) antibodies (Supplementary Table S2). We separately evaluated the B-cell subsets including total B cells, immature B cells, memory B cells, and plasmablasts (PB)/plasma cells (PC), which were phenotypically defined as CD19+, CD19+CD27–CD38+, CD19+CD27+CD38–, and CD19+CD27+CD38+ cells, respectively, in the population gated on total lymphocytes (Fig. 1A). In each B-cell subset, expression of BAFF/APRIL receptors including BAFF-R, TACI, and BCMA was analyzed by evaluating their median fluorescence intensity (MFI). The positive signals of BAFF-R, TACI, or BCMA were determined based on the background level of fluorescence minus one (FMO) control in each B-cell subset. Stained cells were acquired using a FACSCanto II flow cytometer (BD Bioscience), and the acquired data were analyzed using FlowJo software version 10.5.3 (Tree Star Inc., Ashland, OR, USA).

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were stored at −80 °C until ELISA was performed. We used ELISA kits for measuring serum concentrations of BAFF (R&D system, Minneapolis, MN, USA), APRIL (eBioscience, Vienna, Austria), interleukin (IL)-4, IL-6, IL-10, and IL-13 (R&D system) (Supplementary Table S2).

Statistical analysis

The Kolmogorov–Smirnov test was preliminarily performed for estimating the distribution of the data. All data are presented as the median with interquartile range (IQR). P-values of less than 0.05 were defined as statistically significant. The Mann–Whitney U test and Fisher's exact probability test were used to compare two independent groups. The Kruskal–Wallis test was performed to compare the three independent groups, and the Steel–Dwass test was subsequently used for multiple comparison tests. The Spearman's rank correlation coefficient test was performed to evaluate the relationships between serum BAFF or APRIL levels and the expression of each BAFF/APRIL receptor, between clinical findings and serum levels of each cytokine, or between clinical findings and the expression of each BAFF/APRIL receptor. Univariate linear regression analyses were used to evaluate the associations with relevant factors in the expression of circulating B-cell subsets, and we estimated a partial regression coefficient (coefficient) together with a 95% confidence interval (CI). Statistical analyses were performed using JMP software version 14.3.0 (SAS Institute Inc., Cary, NC, USA) and BellCurve for Excel (SSRI, Tokyo, Japan).

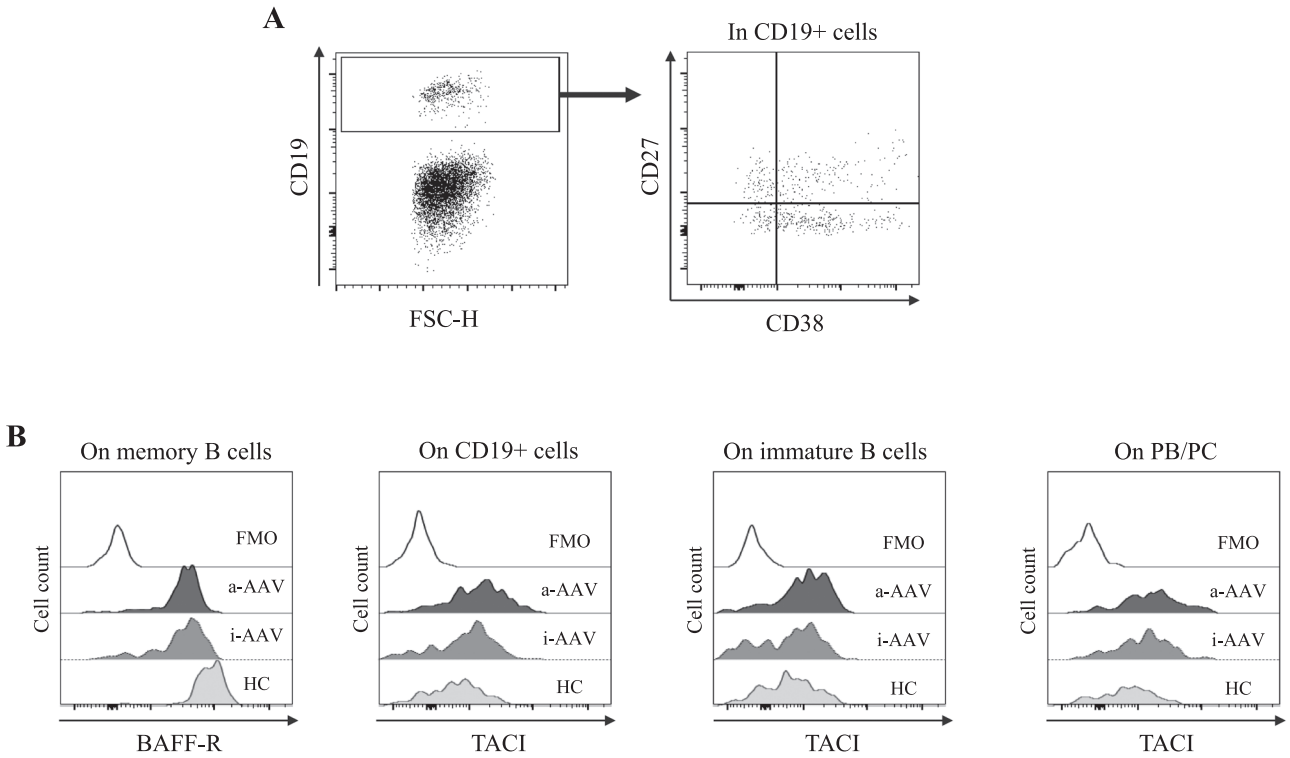

Figure 1: Population of B cell subsets and their expressing BAFF receptor (BAFF-R) or transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI). (A) Representative dot plots of CD19, CD27, and CD38. (B) Representative histograms of BAFF-R on memory B cells, TACI on CD19+ cells, immature B cells, and plasmablasts (PB)/plasma cells (PC) between patient with active ANCA-associated vasculitis (a-AAV), inactive AAV (i-AAV), and healthy control (HC). FMO, fluorescence minus one

Results

BVAS and laboratory findings between patients with a-AAV and those with i-AAV

Median (IQR) BVAS was 14.0 (11.5–19.8) in patients with a-AAV ($P < 0.0001$) (Supplementary Table S1). The frequency of ANCA positivity was significantly higher in patients with a-AAV ($n = 24$, 100%) than in those with i-AAV ($n = 7$, 53%) ($P = 0.0007$). Notably, MPO-ANCA positivity was significantly higher in those with a-AAV than those with i-AAV ($P = 0.006$). Double-positive for MPO- and PR3-ANCA was observed in one patient with a-AAV. The number of WBC, neutrophils, and serum levels of CRP were significantly higher in patients with a-AAV than those with i-AAV ($P < 0.0001$, $P = 0.0002$, and $P < 0.0001$, respectively).

Frequencies of circulating B cells and serum concentration of related cytokines

The frequencies of CD19+ cells, memory B cells, and PB/PC were significantly higher in patients with a-AAV than in those with i-AAV ($P < 0.0001$, $P = 0.004$, and $P < 0.0001$, respectively), and the frequency of immature B cells was also higher in patients with a-AAV than in those with i-AAV, although not significantly different ($P = 0.071$) (Table 1). In comparing the seven consecutive patients before and after treatment, significant decreases in the frequency of CD19+ cells and PB/PC were observed ($P = 0.028$ and $P = 0.018$, respectively) despite there not being significant differences in those of immature and memory B cells (Supplementary Fig. S1). The frequency of PB/PC was significantly higher in patients with a-AAV than in HC ($P < 0.0001$), whereas the frequencies of CD19+ cells, immature B cells, and memory B cells were not significantly different between patients with a-AAV and HC. The frequencies of CD19+ cells, immature B cells, memory B cells, and PB/PC were significantly lower in patients with i-AAV than in HC ($P = 0.0001$, $P = 0.032$, $P = 0.004$, and $P = 0.0001$, respectively). In patients with i-AAV, the frequencies of CD19+ cells, immature B cells, and PB/PC were significantly lower in those administered RTX treatment than in those who were not treated with RTX ($P = 0.003$) (Supplementary Table S3).

The serum levels of BAFF, APRIL, IL-4, and IL-6 were significantly higher in patients with a-AAV than in HC ($P < 0.0001$) (Table 1). Serum levels of APRIL and IL-6 were also significantly higher in patients with a-AAV than those with i-AAV ($P = 0.003$ and $P = 0.0001$, respectively), while those of BAFF, IL-4, IL-10, and IL-13 were not significantly different between patients with a-AAV and i-AAV. The comparisons before and after treatment in the seven consecutive patients indicated significant decreases in serum levels of APRIL, IL-6, and IL-10 ($P = 0.018$) (Supplementary Fig. S2). Serum levels of BAFF, APRIL, and IL-4 were significantly higher in patients with i-AAV than in HC ($P = 0.0003$, $P = 0.005$, and $P < 0.0001$, respectively). However, serum levels of IL-6, IL-10, and IL-13 were not significantly different between patients with i-AAV and HC. In patients with i-AAV, significantly higher serum levels of BAFF were observed in those given RTX treatment than in those who were not given RTX treatment ($P = 0.034$) despite there not being significant differences in serum levels of APRIL, IL-4, IL-6, IL-10, and IL-13 (Supplementary Table S3).

BAFF-R, TACI, and BCMA expression on B cells

BAFF-R expression on memory B cells was significantly lower in patients with a-AAV and i-AAV than in HC (P = 0.017 and P = 0.016) but not significantly different between patients with a-AAV and i-AAV (Figs. 1B and 2). BAFF-R expression on CD19+ cells, immature B cells, and PB/PC was not significantly different among the three groups. TACI expression was significantly higher in patients with a-AAV and those with i-AAV than in HC on CD19+ cells (P = 0.011 and P = 0.028, respectively), immature B cells (P = 0.014 and P = 0.002, respectively), and PB/PC (P = 0.013 and P = 0.011, respectively), despite there not being significant differences between patients with a-AAV and i-AAV. There were no significant differences in TACI expression on memory B cells among the three groups. BCMA expression on memory B cells was significantly lower in the patients with a-AAV than those with i-AAV (P = 0.048). In comparing the results of the seven consecutive patients before and after treatment, expression of BAFF-R, TACI, and BCMA was not significantly different on all B-cell subsets (data not shown). In patients with i-AAV, those with RTX treatment had significantly lower BAFF-R expression on CD19+ cells, immature B cells, memory B cells, and PB/PC (P = 0.011, P = 0.011, P = 0.031, and P = 0.019, respectively), and lower TACI expression on PB/PC (P = 0.034) than those not given the RTX treatment (Supplementary Fig. S3).

Table 1 : Frequencies of circulating B cells and serum levels of cytokines in patients with AAV and healthy controls

	a-AAV	i-AAV	HC	*P* value		
	(*n* = 24)	(*n* = 13)	(*n* = 19)	a-AAV vs. i-AAV	a-AAV vs. HC	i-AAV vs. HC
In total lymphocytes						
% CD19+ cells	15.8 [10.6–22.6]	5.09 [0.93–7.51]	13.1 [10.9–15.8]	<0.0001	0.148	0.0001
% Immature B cells	7.01 [3.91–11.2]	3.48 [0.66–6.21]	7.32 [5.69–9.02]	0.071	0.967	0.032
% Memory B cells	0.48 [0.24–1.63]	0.09 [0.03–0.12]	0.38 [0.23–1.41]	0.004	0.992	0.004
% Plasmablasts/plasma cells	7.78 [6.72–8.35]	0.81 [0.26–1.35]	2.97 [2.52–4.78]	<0.0001	<0.0001	0.0001
In serum						
BAFF (pg/mL)	580 [374–1145]	260 [134–783]	47.8 [29.3–62.1]	0.234	<0.0001	0.0003
APRIL (ng/mL)	4.33 [2.54–5.42]	1.62 [1.16–2.37]	0.66 [0.42–1.02]	0.003	<0.0001	0.005
IL-4 (pg/mL)	143 [139–146]	145 [141–147]	82.5 [62.2–114]	0.673	<0.0001	<0.0001
IL-6 (pg/mL)	13.6 [11.2–35.2]	2.32 [1.95–3.56]	2.75 [1.91–3.48]	0.0001	<0.0001	0.980
IL-10 (pg/mL)	16.5 [12.6–20.1]	10.1 [8.34–13.9]	11.5 [8.751–19.9]	0.052	0.258	0.778
IL-13 (pg/mL)	248 [242–258]	242 [238–248]	301 [248–417]	0.354	0.185	0.148

Data are presented as median [interquartile range (IQR)].
AAV: ANCA-associated vasculitis; a-AAV: active AAV group; i-AAV: inactive AAV group; HC: healthy controls.
P < 0.05 was considered statistically significant.

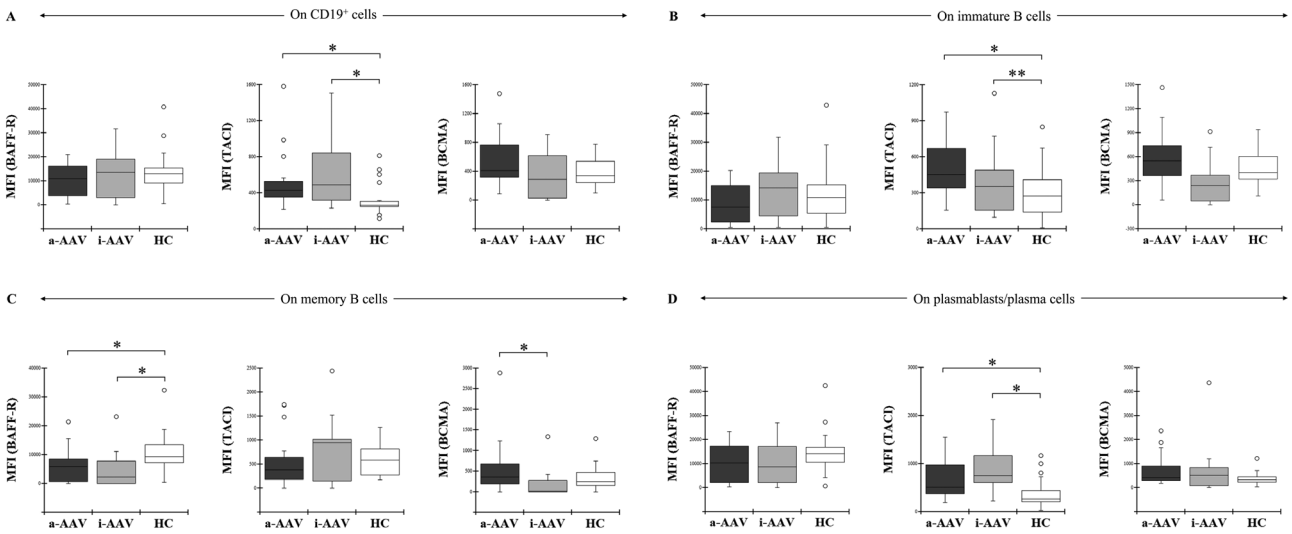

Figure 2: Comparisons of BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA) expression on each B-cell subset between patients with active ANCA-associated vasculitis (a-AAV), inactive AAV (i-AAV), and healthy controls (HC). (A) Median fluorescence intensity (MFI) of BAFF-R, TACI, and BCMA on CD19+ cells, (B) immature B cells, (C) memory B cells, and (D) plasmablasts/plasma cells. *P < 0.05, **P < 0.005

Correlations between serum BAFF or APRIL levels and BAFF/APRIL receptors

Serum levels of APRIL had a weak inverse correlation with TACI expression on CD19+ cells in patients with a-AAV (P = 0.033), and other significant correlations were not found (Table 2). In analyzing patients with i-AAV, serum BAFF levels were significantly inversely correlated with BAFF-R expression on CD19+ cells, immature B cells, and PB/PC (P = 0.005, P = 0.006, and P = 0.006, respectively). Serum levels of APRIL were inversely correlated with TACI expression on memory B cells (P = 0.028).

Table 2: Correlations between serum BAFF/APRIL and their receptors in AAV

	vs. serum BAFF		vs. serum APRIL	
	Coefficient	*P* value	Coefficient	*P* value
In patients with a-AAV (n = 24)				
On CD19+ cells				
MFI-BAFF-R	−0.367	0.077	0.229	0.282
MFI-TACI	−0.218	0.304	−0.438	0.033
MFI-BCMA	−0.205	0.337	0.068	0.754
On immature B cells				
MFI-BAFF-R	−0.247	0.245	0.121	0.572
MFI-TACI	−0.118	0.585	−0.285	0.177
MFI-BCMA	−0.167	0.436	−0.112	0.602
On memory B cells				
MFI-BAFF-R	−0.167	0.436	0.109	0.613
MFI-TACI	−0.259	0.222	−0.168	0.433
MFI-BCMA	0.001	0.995	0.061	0.776
On plasmablasts/plasma cells				
MFI-BAFF-R	−0.197	0.357	0.249	0.241
MFI-TACI	−0.085	0.692	−0.307	0.145
MFI-BCMA	−0.248	0.242	−0.022	0.920
In patients with i-AAV (n = 13)				
On CD19+ cells				
MFI-BAFF-R	−0.725	0.005	0.121	0.694
MFI-TACI	0.511	0.074	−0.193	0.528
MFI-BCMA	0.357	0.231	−0.355	0.234
On immature B cells				
MFI-BAFF-R	−0.714	0.006	0.066	0.830
MFI-TACI	0.382	0.197	−0.240	0.430
MFI-BCMA	0.181	0.553	−0.234	0.442
On memory B cells				
MFI-BAFF-R	−0.355	0.233	−0.214	0.482
MFI-TACI	−0.113	0.712	−0.607	0.028
MFI-BCMA	−0.520	0.069	0.280	0.354
On plasmablasts/plasma cells				
MFI-BAFF-R	−0.714	0.006	0.303	0.315
MFI-TACI	−0.313	0.297	0.311	0.301
MFI-BCMA	0.308	0.306	−0.401	0.175

Spearman's rank correlation coefficient test was conducted for statistical analysis.

Coefficient: correlation coefficient; MFI: median fluorescence intensity.

$P < 0.05$ was considered statistically significant.

Implications of serum BAFF, APRIL, and BAFF/APRIL receptors in circulating B cell expression

Next, we investigated the impacts of serum BAFF, APRIL, IL-4, IL-6, IL-10, IL-13, and BAFF/APRIL receptors on the expression of circulating B cells using univariate linear regression analyses in patients with a-AAV. The frequency of memory B cells was positively associated with serum levels of APRIL (coefficient 0.071, 95% CI 0.035–0.106, P = 0.0004) and their BAFF-R expression (coefficient 1×10^{-4}, 95% CI 6×10^{-5} to 2×10^{-4}, P = 0.002) (Table 3). In univariate linear regression analyses focusing on the frequency of circulating B cells from patients with i-AAV, BAFF-R expression was positively associated with the frequency of CD19+ cells (coefficient 3×10^{-4}, 95% CI 1×10^{-4} to 5×10^{-4}, P = 0.020), immature B cells (coefficient 2×10^{-4}, 95% CI 2×10^{-5} to 4×10^{-4}, P = 0.032), and PB/PC (coefficient 6×10^{-5}, 95% CI 7×10^{-6} to 1×10^{-4}, P = 0.029) (Table 4). Meanwhile, RTX treatment was inversely associated with the frequency of CD19+ cells (coefficient −6.903, 95% CI −10.54 to −3.266, P = 0.002), immature B cells (coefficient −5.373, 95% CI −8.466 to −2.280, P = 0.003), and PB/PC (coefficient −1.123, 95% CI −1.919 to −0.326, p = 0.010). In addition, we investigated the impacts of circulating B-cell subsets on the expression of serum BAFF, APRIL, IL-4, IL-6, IL-10, IL-13, and their BAFF/APRIL receptors using univariate linear regression analyses (Supplementary Tables S4 and S5). Memory B cells were also positively associated with serum APRIL levels (coefficient 6.165, 95% CI 3.055–9.275, P = 0.0004) and their BAFF-R expression (coefficient 2445, 95% CI 1015–3876, P = 0.002) in patients with a-AAV. The frequency of CD19+ cells, immature B cells, and PB/PC were also positively associated with their BAFF-R expression (CD19+ cells: coefficient 1487, 95% CI 288.9–2685, P = 0.020; immature B cells: coefficient 1761, 95% CI 179.4–3342, P = 0.032; PB/PC: coefficient 6440, 95% CI 809.4–12071, P = 0.029).

Relationship between clinical findings and serum cytokines, circulating B cells, or their BAFF/APRIL receptors

We investigated the association between serum levels of BAFF, APRIL, IL-4, IL-6, IL-10, IL-13, the frequencies of circulating B cells, and their expression of BAFF/APRIL receptors in the clinical findings and BVAS. The number of WBC and neutrophils were positively correlated with serum levels of BAFF (P = 0.013 and P = 0.033), IL-6 (P = 0.004 and P = 0.003), IL-10 (P = 0.004 and P = 0.023), IL-13 (P = 0.003 and P = 0.003) (Table 5). TACI expression on immature B cells and BCMA expression on memory B cells were positively correlated with eGFR (P = 0.031 and P = 0.046, respectively), and TACI expression on memory B cells was inversely correlated with the number of neutrophils (P = 0.034) (Table 6). In addition, we compared the frequencies of B-cell subsets, the expression of their BAFF/APRIL receptors, serum levels of BAFF, APRIL, IL-4, IL-6, IL-10, and IL-13 between a-AAV patients with and without symptoms based on BVAS (Supplementary Fig. S4). The frequency of CD19+ cells was significantly higher in patients with fever than in those without fever (20.9 ± 8.7 vs. 13.4 ± 4.7 %, P = 0.018). TACI expression on CD19+ cells and BCMA expression on memory B cells were significantly higher in patients with ENT involvement than in those without it (P = 0.024 and P = 0.026, respectively). BAFF-R expression on CD19+ cells, memory cells, and PB/PC was also

Table 3. Cofactors impacting frequency of target circulating B-cell subsets in patients with active AAV

Cofactors	Target B-cell subsets											
	% CD19+ cells			% Immature B cells			% Memory B cells			% Plasmablasts/Plasma cells		
	Coefficient	[95% CI]	*P* value	Coefficient	[95% CI]	*P* value	Coefficient	[95% CI]	*P* value	Coefficient	[95% CI]	*P* value
Age	0.145	[−0.099, 0.391]	0.232	0.132	[−0.052, 0.315]	0.151	−0.004	[−0.044, 0.036]	0.847	0.028	[−0.051, 0.107]	0.469
Sex	−1.693	[−9.000, 5.614]	0.636	−1.931	[−7.433, 3.571]	0.474	0.094	[−1.067, 1.255]	0.864	0.781	[−1.526, 3.088]	0.489
BVAS	−0.019	[−0.499, 0.463]	0.397	−0.061	[−0.425, 0.302]	0.730	0.009	[−0.067, 0.085]	0.802	0.076	[−0.073, 0.225]	0.301
In serum												
BAFF	0.0001	[−0.006, 0.006]	0.960	−0.0005	[−0.005, 0.004]	0.818	−0.0003	[−0.001, 0.0006]	0.447	−0.0002	[−0.002, 0.002]	0.792
APRIL	0.272	[−0.001, 0.546]	0.051	0.026	[−0.201, 0.252]	0.817	0.071	[0.035, 0.106]	0.0004	0.036	[−0.058, 0.130]	0.434
IL-4	−0.007	[−0.021, 0.007]	0.315	−0.007	[−0.017, 0.004]	0.214	−0.0007	[−0.003, 0.002]	0.520	0.0005	[−0.004, 0.005]	0.829
IL-6	0.001	[−0.065, 0.067]	0.971	−0.014	[−0.064, 0.035]	0.555	−0.002	[−0.012, 0.009]	0.773	0.005	[−0.016, 0.026]	0.624
IL−10	−0.004	[−0.011, 0.004]	0.335	−0.003	[−0.009, 0.003]	0.263	−0.0004	[−0.002, 0.0008]	0.483	0.0003	[−0.002, 0.003]	0.825
IL-13	−0.001	[−0.004, 0.002]	0.361	−0.001	[−0.003, 0.001]	0.320	−0.0002	[−0.0006, 0.0003]	0.474	0.0001	[−0.0008, 0.001]	0.841
On target B-cell subset												
MFI-BAFF-R	0.0001	[−0.0004, 0.0006]	0.703	0.0002	[−0.0002, 0.0006]	0.371	0.0001	[0.00006, 0.0002]	0.002	0.00001	[−0.0001, 0.0002]	0.867
MFI-TACI	−0.003	[−0.015, 0.010]	0.681	−0.006	[−0.017, 0.005]	0.256	−0.0002	[−0.001, 0.0009]	0.699	−0.001	[−0.003, 0.0009]	0.285
MFI-BCMA	−0.002	[−0.013, 0.009]	0.748	−0.002	[−0.010, 0.006]	0.583	−0.00004	[−0.001, 0.0009]	0.928	−0.0005	[−0.025, 0.001]	0.583

Univariate regression analyses were used for statistical analysis.

BAS: the Birmingham Vasculitis Activity Score; CI: confidence interval; MFI: median fluorescence intensity.

$P < 0.05$ was considered statistically significant.

Table 4. Cofactors impacting frequency of target circulating B cell subsets in patients with inactive AAV

Cofactors	Target B-cell subsets											
	% CD19+ cells			% Immature B cells			% Memory B cells			% Plasmablasts/plasma cells		
	Coefficient	[95% CI]	*P*value	Coefficient	[95% CI]	*P* value	Coefficient	[95% CI]	*P* value	Coefficient	[95% CI]	*P* value
Age	−0.108	[−0.307, 0.092]	0.259	−0.092	[−0.251, 0.068]	0.234	−0.031	[−0.068, −0.006]	0.095	−0.005	[−0.045, 0.034]	0.774
Sex	−2.411	[−7.724, 2.903]	0.339	−2.165	[−6.406, 2.076]	0.285	−0.421	[−1.491, 0.649]	0.405	−0.242	[−1.264, 0.781]	0.613
RTX	−6.903	[−10.54, −3.266]	0.002	−5.373	[−8.466, −2.280]	0.003	0.651	[−0.432, 1.734]	0.213	−1.123	[−1.919, −0.326]	0.010
In serum												
BAFF	−0.003	[−0.009, 0.003]	0.245	−0.002	[−0.007, 0.003]	0.307	0.001	[−0.0001, 0.0021]	0.081	−0.0009	[−0.002, 0.0002]	0.093
APRIL	0.464	[−1.662, 2.591]	0.640	0.299	[−1.423, 2.022]	0.709	−0.218	[−0.622, 0.184]	0.258	0.184	[−0.198, 0.565]	0.313
IL−4	0.002	[−0.0002, 0.004]	0.082	0.001	[−0.00009, 0.003]	0.063	−0.000004	[−0.0004, 0.0004]	0.984	0.0001	[−0.0003, 0.0005]	0.584
IL−6	0.040	[−0.008, 0.088]	0.092	0.035	[−0.004, 0.073]	0.072	−0.0002	[−0.011, 0.011]	0.976	0.002	[−0.008, 0.013]	0.619
IL−10	0.002	[−0.0003, 0.004]	0.081	0.002	[−0.0001, 0.003]	0.063	−0.000006	[−0.0005, 0.0005]	0.980	0.0001	[−0.0003, 0.0006]	0.582
IL−13	0.001	[−0.0001, 0.0024]	0.078	0.0009	[−0.0001, 0.002]	0.062	−0.000006	[−0.0003, 0.0003]	0.965	0.0001	[−0.0002, 0.0003]	0.561
On target B-cell subset												
MFI-BAFF-R	0.0003	[0.0001, 0.0005]	0.020	0.0002	[0.0002, 0.0004]	0.032	−0.00002	[−0.0001, 0.0001]	0.582	0.00006	[0.000007, 0.0001]	0.029
MFI-TACI	−0.003	[−0.009, 0.004]	0.407	−0.006	[−0.012, 0.0008]	0.079	0.0002	[−0.0006, 0.001]	0.546	0.0002	[−0.0008, 0.001]	0.686
MFI-BCMA	−0.006	[−0.014, 0.022]	0.133	−0.004	[−0.012, 0.004]	0.289	−0.0004	[−0.002, 0.001]	0.591	−0.0002	[−0.0006, 0.0003]	0.363

Univariate regression analyses were used for statistical analysis.
CI: confidence interval; MFI: median fluorescence intensity; RTX: rituximab.
$P < 0.05$ was considered statistically significant.

Table 5. Correlations between serum cytokines and clinical findings

vs. serum BAFF	vs. serum APRIL		vs. serum IL-4		vs. serum IL-6		vs. serum IL-10		vs. serum IL-13	
	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*p* value
BVAS	–0.379	0.068	0.359	0.084	0.085	0.693	0.013	0.952	–0.293	0.165	–0.090	0.674
Laboratory findings												
White blood cells	0.497	0.013	0.234	0.271	0.154	0.472	0.562	0.004	0.572	0.004	0.581	0.003
Neutrophils	0.437	0.033	0.245	0.248	0.146	0.506	0.568	0.003	0.461	0.023	0.582	0.003
Lymphocytes	0.287	0.174	–0.309	0.139	0.006	0.979	0.006	0.979	0.509	0.011	0.125	0.562
C-reactive protein	0.338	0.106	0.137	0.525	0.107	0.619	0.435	0.034	0.284	0.179	0.219	0.305
eGFR	0.241	0.256	–0.311	0.139	–0.267	0.207	–0.266	0.209	0.235	0.269	0.131	0.541

Spearman's rank correlation coefficient test was conducted for statistical analysis.
Coefficient: correlation coefficient; BAS: the Birmingham Vasculitis Activity Score; eGFR: estimated glomerular filtration rate.
$P < 0.05$ was considered statistically significant.

significantly higher in patients with pulmonary involvement than in those without it ($P = 0.024$, $P = 0.042$, and $P = 0.043$, respectively). Patients with nervous system involvement had significantly lower serum levels of BAFF than those without it (537 ± 674 vs. 1068 ± 397 pg/mL, $P = 0.002$). Other comparisons were not significantly different (data not shown).

Discussion

In the active phase of AAV, significant increases in the proportion of PB/PC were observed. An increase in circulating PB and PC has also been demonstrated in AAV [22, 23], leading to the production of ANCA in a subset of PC. Meanwhile, our results ultimately demonstrated lower proportions of circulating B cells in all subsets from patients with i-AAV than in HC. A reduction in circulating B cells was found as a therapeutic effect in patients in AAV remission [24]. In fact, we evaluated the proportion of circulating B cells in patients with i-AAV using blood samples that were provided during maintenance therapies with immunosuppressive agents. Although monitoring circulating B cells is found to be controversial for predicting prognosis after treatment [25, 26], it was suggested that an increase in PB/PC can be a biological feature in the active phase of AAV.

Serum concentrations of BAFF, APRIL, and other cytokines, including IL-4, IL-6, IL-10, and IL-13 can be implicated in the differentiation of B-cell lineages [27–30]. Consistent with previous studies [12–15, 31], significant increases in BAFF, APRIL, IL-4, and IL-6 were observed in patients with a-AAV. In addition, significant increases in serum levels of BAFF and APRIL remained in the patients with i-AAV; notably, serum levels of BAFF were not significantly different between a-AAV and i-AAV. Persisting high serum levels of BAFF and APRIL after immune suppressive therapies under clinical remission have been demonstrated in not only AAV [14, 15, 32, 33] but also in other autoimmune diseases [11, 34]. It has been postulated that upregulation of BAFF and APRIL may be induced to promote the repopulation of circulating B cells [32], and a process that may be dependent on residual pathological B cells in the tissues [33]. Accordingly, increases in serum levels of BAFF and APRIL may be used as general indicators for monitoring remission in autoimmune diseases, including AAV, in which B-cell lineage is implicated in their pathogenesis. Conversely, the activation of immunocompetent cells, which persistently produce BAFF and APRIL, can participate in AAV relapse. Patients with i-AAV also demonstrated significantly higher concentrations of IL-4 than HC in this study, although no differences have been found in the concentrations of IL-4, IL-10, or IL-13 between patients with AAV and HC [41]. Cytokines categorized as helper T cell type 2, including IL-4, have also been found to increase in patients in AAV remission, suggesting an increase in serum IL-4 levels as a sign of potential indicator in the remission phase of the disease [42].

One feature of BAFF/APRIL receptors in AAV is that higher expression of TACI on CD19+ cells, immature B cells, and PB/PC remained even in patients with i-AAV. TACI, which equally interacts with both BAFF and APRIL [16], is expressed on all B-cell subsets, especially in the marginal zone to mature B cells [10, 43]. Moreover, TACI can be broadly implicated in the generation of B lineage cells. Additionally, increases in B cells were induced in TACI-deficient mice,

Table 6. Correlations between BAFF/APRIL receptors on circulating B-cell subsets and clinical findings

	vs. % CD19+ cells		On CD19+ cells					
			vs. MFI-BAFF-R		vs. MFI-TACI		vs. MFI-BCMA	
	Coefficient	P value	Coefficient	P value	Coefficient	P value	Coefficient	P value
BVAS	−0.039	0.856	0.383	0.065	0.116	0.589	0.328	0.117
Laboratory findings								
White blood cells	−0.050	0.816	0.150	0.483	−0.289	0.171	0.021	0.728
Neutrophils	0.028	0.897	0.197	0.357	−0.213	0.515	0.139	0.703
Lymphocytes	−0.329	0.117	−0.333	0.112	−0.232	0.275	−0.382	0.066
C-reactive protein	0.148	0.489	0.032	0.883	−0.208	0.329	0.129	0.546
eGFR	−0.017	0.936	−0.181	0.399	0.289	0.170	0.067	0.754
	vs. % Immature B cells		vs. MFI-BAFF-R		On immature B cells			
					vs. MFI-TACI		vs. MFI-BCMA	
	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value
BVAS	−0.154	0.470	0.176	0.411	0.182	0.394	0.357	0.087
Laboratory findings								
White blood cells	−0.088	0.683	0.021	0.921	−0.026	0.904	0.022	0.920
Neutrophils	−0.079	0.713	0.035	0.870	0.052	0.809	0.131	0.541
Lymphocytes	−0.119	0.579	−0.265	0.211	−0.243	0.253	−0.203	0.340
C-reactive protein	−0.024	0.912	−0.159	0.459	0.004	0.986	0.027	0.902
eGFR	0.100	0.642	−0.274	0.195	0.441	0.031	0.210	0.325
	vs. % Memory B cells		On memory B cells					
			vs. MFI-BAFF-R		vs. MFI-TACI		vs. MFI-BCMA	
Coefficient		*P* value	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value
BVAS	−0.170	0.426	0.223	0.295	−0.210	0.325	0.139	0.516
Laboratory findings								
White blood cells	0.018	0.932	−0.116	0.590	−0.380	0.067	−0.282	0.182
Neutrophils	0.044	0.839	−0.071	0.741	−0.435	0.034	−0.259	0.223
Lymphocytes	−0.040	0.853	−0.195	0.362	0.040	0.854	−0.258	0.224
C-reactive protein	0.164	0.443	−0.221	0.299	−0.235	0.270	−0.183	0.391
eGFR	−0.162	0.449	−0.183	0.393	0.046	0.831	0.410	0.046

Table 6. Continued

	vs. % Plasmablasts/plasma cells		On Plasmablasts/plasma cells					
			vs. MFI-BAFF-R		vs. MFI-TACI		vs. MFI-BCMA	
	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value	Coefficient	*P* value
BVAS	0.119	0.579	0.338	0.106	–0.126	0.556	–0.019	0.928
			Laboratory findings					
White blood cells	0.109	0.610	0.127	0.556	–0.236	0.268	–0.014	0.949
Neutrophils	0.137	0.525	0.201	0.345	–0.230	0.249	0.006	0.979
Lymphocytes	–0.062	0.774	–0.348	0.096	–0.050	0.815	–0.131	0.542
C-reactive protein	0.280	0.185	–0.011	0.959	–0.179	0.403	0.237	0.265
eGFR	–0.100	0.642	–0.112	0.602	0.318	0.130	0.206	0.334

Spearman's rank-sum coefficient test was conducted for statistical analysis.
BAS: the Birmingham Vasculitis Activity Score; Coefficient: correlation coefficient; eGFR: estimated glomerular filtration rate.
$P < 0.05$ was considered statistically significant.

proposing that TACI can also play a role in negatively regulating proliferation of B cells [6, 43–45]. Considering these properties of TACI on the B-cell lineage, hyperexpression of TACI may be elicited in response to persistently increased serum BAFF and APRIL in patients with a-AAV. Meanwhile, persistently high expression of TACI could contribute to the reduction of circulating B cells along with attenuated interaction of other B-cell inducers such as IL-6 in patients with i-AAV. Another feature of BAFF/APRIL receptors in AAV was reduced expression of BAFF-R on memory B cells. BAFF-R, which is strongly bound to BAFF and promotes multiple intracellular signaling such as NK-κB, MAPK, and/or PI3K/Akt pathways [17, 46–48], is crucial for the survival and maturation of B cells [6, 49]. The expression of BAFF-R can broadly mediate differentiation of B cells from pre-B cell generation and is also implicated in the survival and maturation of translational to follicular or marginal zone B cell populations [47, 50, 51]. Notably, functions of BAFF-R are pivotal for the generation of memory B cell subset [17]. In contrast, persistent exposure to BAFF was found to suppress BAFF-R expression even in healthy individuals [18]. Considering the affinity of BAFF-R to memory B cells and the sensitivity of BAFF-R to BAFF, decreased expression of BAFF-R on memory B cells may be ultimately driven by persistent exposure to high concentrations of BAFF. Inverse correlations between serum BAFF levels and BAFF-R expression on CD19+ cells, immature B cells, and PB/PC were also demonstrated in the i-AAV group, suggesting that BAFF-R expression might be a more likely physiological response depending on serum concentration of BAFF in remission, whereas decreased expression of BAFF-R remained on memory B cells. Moreover, significantly low expression of BAFF-R on all B-cell subsets and higher concentrations of BAFF in patients given RTX treatment may support the crosstalk interaction between BAFF and BAFF-R in the B-cell lineage signals.

Univariate regression analyses demonstrated positive associations of the proportion of memory B cells with BAFF-R expression and serum APRIL levels in the patients with a-AAV. The differentiation of PC from memory B cells was found to be dependent on the concentration of APRIL in an *in vitro* study and some autoimmune diseases [53, 54], suggesting that APRIL can promote differentiation of memory B cells into PC-secreting autoantibodies as an antigen-independent action. In addition, BAFF-R expression may be a positive regulating factor for inducing memory B cells in the active phase of AAV. Meanwhile, our results suggested that BAFF-R expression was positively associated with the proportions of CD19+ cells, immature B cells, and PB/PC in patients in AAV remission.

BAFF-R expression on B cells, including memory and immature B cells, was also significantly decreased in the patients with SJS compared to healthy individuals [18, 52], whilst there was no significant difference between patients with SLE and healthy individuals [18, 19]. Our patients with AAV demonstrated increased expression of TACI on B cells; conversely, reduced expression of TACI was observed in patients with SLE [19]. Accordingly, it has been suggested that the features of BAFF/APRIL receptors expression are not uniformly shown among different types of diseases, even in those in which serum BAFF and APRIL levels increase in a similar manner.

Our results suggest that halting the increase in BAFF and APRIL serum levels using some practical antagonists, such as belimumab, blisibimod, tabalumab, or atacicept [35–38], may prevent AAV relapse as same as SLE. However, concomitant use of belimumab under maintenance therapy with azathioprine and corticosteroid did not reduce relapse in AAV [39], despite being effective in combination with RTX [39, 40]. Given the different features of BAFF/APRIL receptors expression between AAV and SLE as well as the results of these clinical trials, facilitated APRIL and TACI pathways might be more relevant for the pathogenesis of AAV than other diseases.

There were some limitations in this study. First, more extensive immune system features, including antigen presentation, immunocompetent cell-contacts, and stimulation of other cytokines, can affect the differentiation of B cells than domains investigated in our study. Therefore, it is necessary to establish an experimental system that investigates the independent roles of BAFF and APRIL signaling for B-cell lineage with and without several confounding factors in the immune system. Second, we had an insufficient number of patients with i-AAV to analyze the implication of experimental results in the symptoms and clinical course, although some symptoms were significantly increased in the patients presenting with ear, nose, and throat or pulmonary manifestations. It is necessary to longitudinally accumulate blood samples and clinical information from a large number of patients to determine the usefulness of B-cell subsets, BAFF/APRIL receptors, or related cytokines as biomarkers for evaluating clinical findings in AAV. Third, we had a very small number of patients; therefore, we ultimately performed exploratory and univariate analyses in this study. However, multiple linear regression analyses are necessary for statistically analyzing their relationship in larger sample size for determining more precise crosstalk interaction between circulating B-cell subsets, their expressing BAFF/APRIL receptors, and serum levels of BAFF and APRIL in the pathogenesis of AAV.

In conclusion, significantly increased proportion of PB/PC and serum levels of BAFF and APRIL were observed in patients with a-AAV. Increased serum levels of BAFF and APRIL remained, whereas a significantly decreased proportion of all B-cell subsets was observed, in patients with i-AAV. Increased expression of TACI on CD19+ cells, immature B cells, and PB/PC, as well as decreased expression of BAFF-R on memory B cells, were demonstrated as the significant features in both patients with a-AAV and those with i-AAV. Patients with a-AAV had the positive associations of serum APRIL levels and BAFF-R expression with the proportion of memory B cells. In patients with i-AAV, BAFF-R expression indicated the positive associations with the proportions of CD19+ cells, immature B cells, and PB/PC. Our results suggested a pivotal implication in the generation of B cells related to the pathogenesis of AAV. Nevertheless, it is necessary to elucidate a more precise mechanism of BAFF/APRIL signaling for finding therapeutic targets and establishing useful biomarkers in AAV.

Supplementary data

Supplementary data is available at *Clinical and Experimental Immunology* online.

Acknowledgements

We thank all members of the Department of Medicine (Neurology and Rheumatology) at Shinshu University Hospital for treating the patients who participated in the study. We would like to thank Editage (www.editage.com) for English language editing.

Ethics approval

The local ethics committee of Shinshu University approved this study (approval number: 4390).

Conflict of interests

The authors declare that they have no financial or personal conflicts of interest

Funding

This study was supported by JSPS KAKENHI Grant Number JP21K08455.

Data availability

The data for the analyses in this study are available from the corresponding author upon reasonable request.

Patient consent

All participants provided written informed consent.

Author contributions

All authors were involved in drafting the article or critically revising it for important intellectual content, and all authors approved the final manuscript. Y-Shi designed this study and developed the structure and argument for this study. All authors recruited blood samples and clinical data. Y-Shi performed laboratory investigations and analyzed obtained data.

References

1. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013, 65, 1–11.
2. Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat Rev Rheumatol* 2014, 10, 463–73.
3. Sciascia S, Ponticelli C, Roccatello D. Pathogenesis-based new perspectives of management of ANCA-associated vasculitis. *Autoimmun Rev* 2022, 21, 103030.
4. Nakazawa D, Masuda S, Tomaru U, Ishizu A. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. *Nat Rev Rheumatol* 2019, 15, 91–101.
5. Shimojima Y, Kishida D, Ichikawa T, Takamatsu R, Nomura S, Sekijima Y. Oxidative stress promotes instability of regulatory T cells in antineutrophil cytoplasmic antibody-associated vasculitis. *Front Immunol* 2021, 12, 789740.
6. 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, 203–15.
7. Tangye SG, Bryant VL, Cuss AK, Good KL. BAFF, APRIL and human B cell disorders. *Semin Immunol* 2006, 18, 305–17.

8. Mackay F, Schneider P. Cracking the BAFF code. *Nat Rev Immunol* 2009, 9, 491–502.
9. Seyler TM, Park YW, Takemura S, Bram RJ, Kurtin PJ, Goronzy JJ, et al. BLyS and APRIL in rheumatoid arthritis. *J Clin Invest* 2005, 115, 3083–92.
10. Shabgah AG, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohammadi M. The role of BAFF and APRIL in rheumatoid arthritis. *J Cell Physiol* 2019, 234, 17050–63.
11. Pollard RP, Abdulahad WH, Vissink A, Hamza N, Burgerhof JG, Meijer JM, et al. Serum levels of BAFF, but not APRIL, are increased after rituximab treatment in patients with primary Sjogren's syndrome: data from a placebo-controlled clinical trial. *Ann Rheum Dis* 2013, 72, 146–8.
12. Shimojima Y, Kishida D, Sekijima Y. Increased BAFF and APRIL levels in the cerebrospinal fluid of patients with anti-neutrophil cytoplasmic antibody-related hypertrophic pachymeningitis. *Cytokine* 2017, 99, 305–9.
13. Krumbholz M, Specks U, Wick M, Kalled SL, Jenne D, Meinl E. BAFF is elevated in serum of patients with Wegener's granulomatosis. *J Autoimmun* 2005, 25, 298–302.
14. Nagai M, Hirayama K, Ebihara I, Shimohata H, Kobayashi M, Koyama A. Serum levels of BAFF and APRIL in myeloperoxidase anti-neutrophil cytoplasmic autoantibody-associated renal vasculitis: association with disease activity. *Nephron Clin Pract* 2011, 118, c339–45.
15. Holden NJ, Williams JM, Morgan MD, Challa A, Gordon J, Pepper RJ, et al. ANCA-stimulated neutrophils release BLyS and promote B cell survival: a clinically relevant cellular process. *Ann Rheum Dis* 2011, 70, 2229–33.
16. Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. *Semin Immunol* 2006, 18, 263–75.
17. Sevdali E, Block Saldana V, Speletas M, Eibel H. BAFF receptor polymorphisms and deficiency in humans. *Curr Opin Immunol* 2021, 71, 103–10.
18. Sellam J, Miceli-Richard C, Gottenberg JE, Ittah M, Lavie F, Lacabaratz C, et al. Decreased B cell activating factor receptor expression on peripheral lymphocytes associated with increased disease activity in primary Sjögren's syndrome and systemic lupus erythematosus. *Ann Rheum Dis* 2007, 66, 790–7.
19. Carter RH, Zhao H, Liu X, Pelletier M, Chatham W, Kimberly R, et al. Expression and occupancy of BAFF-R on B cells in systemic lupus erythematosus. *Arthritis Rheum* 2005, 52, 3943–54.
20. Watts R, Lane S, Hanslik T, Hauser T, Hellmich B, Koldingsnes W, et al. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann Rheum Dis* 2007, 66, 222–7.
21. Mukhtyar C, Lee R, Brown D, Carruthers D, Dasgupta B, Dubey S, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis* 2009, 68, 1827–32.
22. Matsumoto K, Suzuki K, Yoshimoto K, Seki N, Tsujimoto H, Chiba K, et al. Significant association between clinical characteristics and immuno-phenotypes in patients with ANCA-associated vasculitis. *Rheumatology (Oxford)* 2020, 59, 545–53.
23. Manrique J, Chan E, Hartzell S, Mon-Wei Yu S, Cantarelli C, Fernandez LF, et al. Circulating B cells, plasma cells, and Treg associate with ANCA levels in ANCA-associated vasculitis. *Kidney Int Rep* 2021, 6, 496–500.
24. Fazekas B, Moreno-Olivera A, Kelly Y, O'Hara P, Murray S, Kennedy A, et al. Alterations in circulating lymphoid cell populations in systemic small vessel vasculitis are non-specific manifestations of renal injury. *Clin Exp Immunol* 2018, 191, 180–8.
25. Charles P, Terrier B, Perrodeau E, Cohen P, Faguer S, Huart A, et al. Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2). *Ann Rheum Dis* 2018, 77, 1143–9.
26. Arnold J, Vital EM, Dass S, Aslam A, Rawstron AC, Savic S, et al. A Personalized rituximab retreatment approach based on clinical and b-cell biomarkers in ANCA-associated vasculitis. *Front Immunol* 2021, 12, 803175.
27. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 2015, 16, 448–57.
28. Schaper F, Rose-John S. Interleukin-6: biology, signaling and strategies of blockade. *Cytokine Growth Factor Rev* 2015, 26, 475–87.
29. Lin YJ, Goretzki A, Schülke S. Immune metabolism of IL-4-activated B cells and Th2 cells in the context of allergic diseases. *Front Immunol* 2021, 12, 790658.
30. Itoh K, Hirohata S. The role of IL-10 in human B cell activation, proliferation, and differentiation. *J Immunol* 1995, 154, 4341–50.
31. Berti A, Warner R, Johnson K, Cornec D, Schroeder D, Kabat B, et al. Brief report: circulating cytokine profiles and antineutrophil cytoplasmic antibody specificity in patients with antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheumatol* 2018, 70, 1114–21.
32. Shimojima Y, Kishida D, Nomura S, Sekijima Y. Cerebrospinal fluid levels of BAFF and APRIL as direct indicators of disease activity in anti-neutrophil cytoplasmic antibody-related hypertrophic pachymeningitis. *Clin Rheumatol* 2020, 39, 3145–8.
33. Tsuboi K, Noguchi K, Kitano M, Furukawa T, Hashimoto T, Azuma N, et al. Serum B cell activating factor (BAFF) as a biomarker for induction of remission with rituximab in ANCA-associated vasculitis. *Immunol Med* 2022, 45, 238–43.
34. Vallerskog T, Heimburger 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, R167.
35. Stohl W, Hiepe F, Latinis KM, Thomas M, Scheinberg MA, Clarke A, et al. Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis Rheum* 2012, 64, 2328–37.
36. Merrill JT, Shanahan WR, Scheinberg M, Kalunian KC, Wofsy D, Martin RS. Phase III trial results with blisibimod, a selective inhibitor of B-cell activating factor, in subjects with systemic lupus erythematosus (SLE): results from a randomised, double-blind, placebo-controlled trial. *Ann Rheum Dis* 2018, 77, 883–9.
37. Merrill JT, van Vollenhoven RF, Buyon JP, Furie RA, Stohl W, Morgan-Cox M, et al. Efficacy and safety of subcutaneous tabalumab, a monoclonal antibody to B-cell activating factor, in patients with systemic lupus erythematosus: results from ILLUMINATE-2, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study. *Ann Rheum Dis* 2016, 75, 332–40.
38. Dall'Era M, Chakravarty E, Wallace D, Genovese M, Weisman M, Kavanaugh A, et al. Reduced B lymphocyte and immunoglobulin levels after atacicept treatment in patients with systemic lupus erythematosus: results of a multicenter, phase Ib, double-blind, placebo-controlled, dose-escalating trial. *Arthritis Rheum* 2007, 56, 4142–50.
39. Jayne D, Blockmans D, Luqmani R, Moiseev S, Ji B, Green Y, et al. Efficacy and safety of belimumab and azathioprine for maintenance of remission in antineutrophil cytoplasmic aantibody-aassociated vasculitis: a randomized controlled study. *Arthritis Rheumatol* 2019, 71, 952–63.
40. Prendecki M, McAdoo SP. New therapeutic targets in antineutrophil cytoplasm antibody-associated vasculitis. *Arthritis Rheumatol* 2021, 73, 361–70.
41. Schönermarck U, Csernok E, Trabandt A, Hansen H, Gross WL. Circulating cytokines and soluble CD23, CD26 and CD30 in ANCA-associated vasculitides. *Clin Exp Rheumatol* 2000, 18, 457–63.
42. Szczeklik W, Jakieła B, Wawrzycka-Adamczyk K, Sanak M, Hubalewska-Mazgaj M, Padjas A, et al. Skewing toward Treg and Th2 responses is a characteristic feature of sustained remission in ANCA-positive granulomatosis with polyangiitis. *Eur J Immunol* 2017, 47, 724–33.

43. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. *J Allergy Clin Immunol* 2013, 131, 959–71.
44. Seshasayee D, Valdez P, Yan M, Dixit VM, Tumas D, Grewal IS. Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. *Immunity* 2003, 18, 279–88.
45. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T, et al. Activation and accumulation of B cells in TACI-deficient mice. *Nat Immunol* 2001, 2, 638–43.
46. Rauch M, Tussiwand R, Bosco N, Rolink AG. Crucial role for BAFF-BAFF-R signaling in the survival and maintenance of mature B cells. *PLoS One* 2009, 4, e5456.
47. Stadanlick JE, Kaileh M, Karnell FG, Scholz JL, Miller JP, Quinn WJ 3rd, et al. Tonic B cell antigen receptor signals supply an NF-kappaB substrate for prosurvival BLyS signaling. *Nat Immunol* 2008, 9, 1379–87.
48. Woodland RT, Fox CJ, Schmidt MR, Hammerman PS, Opferman JT, Korsmeyer SJ, et al. Multiple signaling pathways promote B lymphocyte stimulator dependent B-cell growth and survival. *Blood* 2008, 111, 750–60.
49. Ng LG, Sutherland AP, Newton R, Qian F, Cachero TG, Scott ML, et al. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. *J Immunol* 2004, 173, 807–17.
50. Meyer-Bahlburg A, Andrews SF, Yu KO, Porcelli SA, Rawlings DJ. Characterization of a late transitional B cell population highly sensitive to BAFF-mediated homeostatic proliferation. *J Exp Med* 2008, 205, 155–68.
51. Warnatz K, Salzer U, Rizzi M, Fischer B, Gutenberger S, Böhm J, et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc Natl Acad Sci U S A* 2009, 106, 13945–50.
52. Neys SFH, Verstappen GM, Bootsma H, Kroese FGM, Hendriks RW, Corneth OBJ. Decreased BAFF receptor expression and unaltered B cell receptor signaling in circulating B cells from primary Sjogren's syndrome patients at diagnosis. *Int J Mol Sci* 2022, 23, 5101.
53. Yeh TW, Okano T, Naruto T, Yamashita M, Okamura M, Tanita K, et al. APRIL-dependent lifelong plasmacyte maintenance and immunoglobulin production in humans. *J Allergy Clin Immunol* 2020, 146, 1109–20.e4.
54. Joo H, Coquery C, Xue Y, Gayet I, Dillon SR, Punaro M, et al. Serum from patients with SLE instructs monocytes to promote IgG and IgA plasmablast differentiation. *J Exp Med* 2012, 209, 1335–48.