Favorable efficacy of rituximab in ANCA-associated vasculitis patients with excessive B cell differentiation

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Abstract

**Objectives:** B-cell depletion by rituximab (RTX) is an effective treatment for anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV). However, peripheral B cell phenotypes and the selection criteria for RTX therapy in AAV remain unclear.

**Methods:** Phenotypic characterization of circulating B cells was performed by 8-color flow cytometric analysis in 54 newly diagnosed AAV patients (20 granulomatosis with polyangiitis and 34 microscopic polyangiitis). Patients were considered eligible to receive intravenous cyclophosphamide pulse (IV-CY) or RTX. All patients also received high-dose glucocorticoids (GC). We assessed circulating B cell phenotypes and evaluated the efficacy after 6 months of treatment.

**Results:** There were no significant differences in the rate of clinical improvement, relapses, or serious adverse events between patients receiving RTX and IV-CY. The proportion of effector or class-switched memory B cells increased in 24 out of 54 patients (44%). The proportions of peripheral T and B cell phenotypes did not correlate with BVAS at baseline. However, among peripheral B cells, the proportion of class-switched memory B cells negatively correlated with the rate of improvement in BVAS at 6 months after treatment initiation ($r = -0.28$, $p = 0.04$). Patients with excessive B cell differentiation were defined as those in whom the proportion of class-switched memory B cells or IgD$^-$CD$^-$ B cells among all B cells was $>2$ SDs higher than the mean in the HCs. The rate of Birmingham Vasculitis Activity Score (BVAS) remission in patients with excessive B cell differentiation was significantly lower than that in patients without. In patients with excessive B cell differentiation, the survival rate, the rate of BVAS remission, and dose reduction of GC were significantly improved in the RTX group compared to those in the IV-CY group after 6 months of treatment.

**Conclusions:** The presence of excessive B cell differentiation was associated with treatment resistance. However, in patients with circulating B cell abnormality, RTX was effective and increased survival compared to IV-CY. The results suggest that multi-color flow cytometry may be useful to determine the selection criteria for RTX therapy in AAV patients.

**Introduction**

Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) is a systemic
autoimmune disease with poor prognosis that damages various organs and commonly occurs in the elderly.[1] To induce remission, AAV is treated with high-dose glucocorticoid (GC) therapy combined with cyclophosphamide (CY), azathioprine (AZA), etc.[2] However, because these immunosuppressants are nonspecific and cause adverse reactions, development of therapeutic agents specific to the pathology of this disease is warranted. The efficacy of B-cell depletion therapy has been demonstrated in AAV patients by the Rituximab in ANCA-Associated Vasculitis (RAVE) trial [3] and the Randomized Trial of Rituximab Versus Cyclophosphamide for ANCA-Associated Renal Vasculitis (RITUXVAS) [4] trial. Rituximab (RTX) has been shown to be as effective as CY for inducing remission in AAV patients. However, it is unknown how B cells actually affect disease activity and prognosis in AAV patients. Furthermore, although RTX, which is the first biologic drug approved for the treatment of AAV, has a specific action mechanism of depleting B cells, the differential use of RTX and CY remains a major clinical question.

The surface phenotypes of peripheral lymphocytes in patients with autoimmune disease are often considered to reflect affected tissue states. We have analyzed peripheral lymphocytes from patients with systemic lupus erythematosus (SLE),[5] rheumatoid arthritis,[6] psoriatic arthritis,[7] using 8-color flow cytometry. In these studies, we showed that analysis of peripheral lymphocytes provides valuable insight into the lineage, differentiation stage, activation state, etc., of lymphocytes associated with pathological conditions and can be an indicator for treatment selection.

Thus, the present study aimed to identify abnormalities in the differentiation of peripheral lymphocytes in AAV patients, analyze the clinical significance of these abnormalities, and investigate how to differentiate the use of RTX and CY according to abnormalities in lymphocytes.

Materials And Methods
Patients
The present study included 54 patients with AAV newly diagnosed by the Watts algorithm [8] who were admitted to our hospital for progressive organ dysfunction (e.g., the lung, kidney, and nervous system) and received high-dose GC therapy between March 2013 and June 2018. Of these patients, remission induction therapy was instituted with RTX in 34 patients and with intravenous CY pulse in
20 patients. The drugs used for remission induction therapy were selected by attending physicians who took baseline patient characteristics into account but remained blinded to B-cell phenotypes. The treatment regimens are shown in Supplementary Method. Moreover, healthy individuals matched for age and sex were included as healthy controls (HCs). The Human Ethics Review Committee of University of Occupational & Environmental Health, Japan reviewed and approved this study, including the collection of peripheral blood samples from HCs and AAV patients. Each subject provided a signed consent form.

Treatments

All patients received high-dose glucocorticoid therapy (prednisolone 1 mg/kg/day) for 4 weeks. The rituximab group received intravenous rituximab (at a dose of 375 mg per square meter of body-surface area once weekly for 4 weeks). The IV-CY group received intravenous cyclophosphamide (at a dose 15 mg/kg at 2-week intervals for 12 weeks). Glucocorticoid dosage was reduced to 10 to 20 percent per 1 to 2 weeks from 4 weeks to 6 months while accommodating individual patient response.

Clinical measurements

Disease activity was assessed by the Birmingham Vasculitis Activity Score (BVAS) 2003 [9] at baseline and 6 months of treatment. BVAS remission defined as BVAS = 0.

Flow cytometric analysis

All patients in this study were enrolled in the FLOW registry, with immunophenotyping analysis performed by multicolor flow cytometry, and the results were recorded in the registry. Peripheral blood (PB) mononuclear cells (PBMCs) were isolated from PB samples using lymphocyte separation medium (ICN/Cappel). Next, PBMCs were resuspended in phosphate-buffered saline (PBS)/3% human IgG (Baxter) in order to block Fc receptors and prevent nonspecific antibody binding, followed by incubation for 15 minutes at 48 °C in the dark. The cells were then washed with PBS containing 1% bovine serum albumin. Background fluorescence was assessed using appropriate isotype- and fluorochrome-matched control monoclonal antibodies. After staining with antibodies, the cells were assessed by multicolor flow cytometry (FACS Verse; BD Biosciences), and the data were analyzed with FlowJo software (Tree Star). Phenotypes of immune cell subsets were defined based on the Human Immunology Project protocol for comprehensive 8-color flow cytometric analysis proposed by the
National Institutes of Health/Federation of Clinical Immunology Societies. Details of the procedure of flow cytometric analysis and gating strategy are shown in Supplementary Table S1 and Fig. 1.

Statistical analysis
Continuous data are expressed as the mean ± standard deviation (SD) and median (range), and categorical data are expressed as n (%). The significance of differences between groups was assessed by Student’s t-tests, and Mann-Whitney U-tests, with Fisher’s exact test used for nominal variables. When comparing among four categories, statistical significance was determined by P < 0.05/4 using Bonferroni method to avoid multiplicity. The Kaplan-Meier method was used to assess the survival rates and the differences were analyzed by the log-rank test.

Results
Patient backgrounds
The mean age of all patients was 70.6 ± 8.3 years, and there were roughly the same number of men and women (male : female = 54:46). All patients had new-onset disease. Microscopic polyangiitis was diagnosed in 34 patients (63.0%) and granulomatosis with polyangiitis in 20 patients (37.0%). For remission induction therapy, the patients were divided into those receiving high-dose GC therapy combined with RTX (RTX group) and those receiving GC therapy combined with intravenous CY pulse (IV-CY group). The baseline patient characteristics in these groups are compared in Table 1. In both groups, the common organ dysfunctions were respiratory dysfunction and nephropathy. BVAS was 17.4 ± 6.5 in the RTX group and 15.1 ± 4.6 in the IV-CY group, showing that disease activity was high in the majority of the patients. Between the RTX and IV-CY groups, no significant differences were observed in age, sex, disease duration, vasculitis type, BVAS, organ dysfunction, or GC dose.
### Table 1
Baseline AAV patient characteristics by treatment group

| Variables                                      | Rituximab group n = 34 | IV-CY group n = 20 | p-value |
|------------------------------------------------|-------------------------|--------------------|---------|
| Age (years)                                    | 70.4 (8.4)              | 70.9 (8.4)         | 0.86    |
| Gender, n (% female)                           | 19 (55.9%)              | 10 (50.0%)         | 0.78    |
| Disease duration (month)                       | 3 (2-5)                 | 4 (1-7)            | 0.93    |
| New onset, n(%)                                | 34 (100.0%)             | 20 (100.0%)        | 1.00    |
| ANCA-positive at diagnosis, n(%)               |                         |                    |         |
| Proteinase 3 ANCA                              | 7 (20.6%)               | 1 (5.0%)           | 0.23    |
| Myeloperoxidase-ANCA                           | 29 (85.3%)              | 19 (95.0%)         | 0.39    |
| ANCA-associated vasculitis type, n(%)          |                         |                    |         |
| MPA                                            | 19 (55.9%)              | 15 (75.0%)         | 0.24    |
| GPA                                            | 15 (44.1%)              | 5 (15.0%)          |         |
| BVAS                                           | 17.4 (6.5)              | 15.1 (4.6)         | 0.15    |
| Organ involvement, n                           |                         |                    |         |
| Cutaneous involvement                         | 2                       | 0                  | 0.52    |
| Mucous membranes and eyes                      | 3                       | 2                  | 1.00    |
| Ear, nose, and throat                          | 11                      | 6                  | 1.00    |
| Pulmonary involvement                          | 27                      | 15                 | 0.74    |
| Renal involvement                              | 28                      | 18                 | 0.75    |
| Neurologic involvement                         | 11                      | 3                  | 0.21    |
| GC dose at base line                           | 56.3 (11.5)             | 61.6 (16.7)        | 0.17    |

Values listed as mean (SD) and median (minimum-maximum) unless otherwise stated. The statistical difference was determined by Student’s t-test and Mann-Whitney’s U test, with chi-square test used for nominal variables. Difference with p < 0.05 was considered significant.

No difference in the rate of remission in BVAS between groups

First, the therapeutic effects of RTX and conventional therapy on AAV were assessed. At 6 months after treatment initiation, BVAS was significantly improved in both the RTX and IV-CY groups (mean change: −14.2 ± 6.3, p < 0.01 in the RTX group; −15.2 ± 5.1, p < 0.01 in the IV-CY group) (Fig. 1A).

The rate of remission in BVAS at 6 months after treatment initiation was not significantly different between the RTX and IV-CY groups [RTX group = 61.8%, IV-CY group: 40.0%, p = 0.16] (Fig. 1B). In addition, no significant differences in the incidences of adverse events, severe infection, leukopenia, or thrombocytopenia or in mortality were observed between the two groups (Table 2). The most common adverse event was infection in both groups, and most deaths were caused by severe infection.
### Table 2
Adverse events by treatment group

| Variables         | Rituximab group n = 34 | IV-CY group n = 20 | p-value |
|-------------------|--------------------------|---------------------|---------|
| All adverse events, no. | 19                       | 14                  | 0.39    |
| Death, no         | 4                        | 4                   | 0.45    |
| Sepsis (pyelonephritis) |                         |                     |         |
| Invasive pulmonary aspergillosis |               |                     |         |
| Bacterial pneumonia |                          |                     |         |
| stroke             |                          |                     |         |
| Leukopenia, no     | 4                        | 4                   | 0.45    |
| Thrombocytopenia, no | 4                        | 2                   | 1.00    |
| Severe infection, no | 11                       | 12                  | 0.09    |
| CMV infection, n = 2 |                          |                     |         |
| Bacterial pneumonia, n = 2 |                  |                     |         |
| Legionella pneumonia |                          |                     |         |
| Sepsis (pyelonephritis) |                         |                     |         |
| Invasive pulmonary aspergillosis |                 |                     |         |
| PCP                |                          |                     |         |
| Invasive pulmonary aspergillosis |                 |                     |         |
| Thrombocytopenia, no | 4                        | 4                   | 0.45    |
| Leukopenia, no     | 4                        | 4                   | 0.45    |
| Severe infection, no | 11                       | 12                  | 0.09    |
| VTE, no            | 1                        | 0                   | 0.37    |
| PCP: Pneumocystis pneumonia, CBV: cytomegalovirus, VTE: venous thromboembolism |

The statistical difference was determined by chi-square test. Difference with p < 0.05 was considered significant.

Increased proportion of IgD<sup>-</sup>CD27<sup>-</sup> double-negative memory B cells in patients with AAV

Phenotypes of peripheral T and B cells before treatment initiation were analyzed by 8-color flow cytometry and compared between AAV patients and 15 HCs matched for age and sex (Fig. 2, Supplementary Table S2). Among cluster of differentiation (CD)-4 T cells, the proportion of naïve CD4 T cells was significantly higher in AAV patients than in HCs, whereas the proportion of central memory CD4 T cells was significantly lower. As for CD8 T cells, no differences in phenotype were observed between AAV patients and HCs. By contrast, in B cells, the proportion of immunoglobulin (Ig)-M memory B cells was significantly lower in AAV patients than in HCs (HC = 19.0 ± 6.9, AAV = 12.1 ± 6.7, p < 0.01), whereas the proportion of IgD<sup>-</sup>CD27<sup>-</sup> double-negative B cells was significantly higher (HC = 5.4 ± 2.7, AAV = 9.8 ± 7.9, p = 0.04).

Next, the association between peripheral CD4<sup>+</sup> T and B cell phenotypes and clinical signs before treatment initiation was analyzed (Fig. 3, Supplementary Table S3). Proportions of peripheral T and B cell phenotypes did not correlate with BVAS at baseline. However, among peripheral B cells, the
proportion of naive B cells positively correlated with the rate of improvement in BVAS at 6 months after treatment initiation ($r = 0.35$, $p < 0.01$), whereas the proportion of class-switched memory B cells negatively correlated with this rate ($r = -0.28$, $p = 0.04$).

**Association of excessive B cell differentiation with treatment resistance**

Next, among the AAV patients, we compared the response to treatment between patients with and without excessive B cell differentiation. Patients with excessive B cell differentiation were defined as those in whom the proportion of class-switched memory B cells or IgD$^-$CD27$^-$ B cells among all B cells was $> 2$ SDs higher than the mean in the HCs (class-switched memory B cells/B cells $> 23\%$ or IgD$^-$CD27$^-$ B cells/B cells $> 11.3\%$) (Fig. 4A). Based on this definition, 24 of the 54 AAV patients (44.4%) showed excessive B cell differentiation (Fig. 4B).

Between the patients with and without excessive B cell differentiation, no notable differences were observed concerning patient characteristics or T cell phenotypes before administration of remission induction therapy (Supplementary Tables S4 and S5). However, after 6 months therapy, BVAS was significantly higher in patients with excessive B cell differentiation than in those without ($p < 0.01$, Fig. 4C), and the rate of remission in BVAS was significantly lower in those with excessive B cell differentiation at 6 months [presence of excessive B cell differentiation = 37.5\%, absence of excessive B cell differentiation = 66.7\%, $p < 0.01$] (Figs. 4D). These results reveal that patients with excessive B cell differentiation are resistant to treatment.

**Favorable effectiveness of RTX in improving BVAS, reducing GC use, and increasing survival among patients with circulating B cell abnormalities**

Lastly, the two treatment groups were further divided according to the presence or absence of excessive B cell differentiation, and responses to treatment were compared among the four resulting treatment groups. No significant differences were observed in disease duration, age, type of vasculitis, BVAS, or GC dose (Table 3). After 6 months of remission induction therapy, patients with excessive B cell differentiation in the IV-CY group had significantly higher BVAS than patients in the other three groups (Figs. 5A and Supplementary Fig. 2A). Meanwhile, patients with excessive B cell differentiation in the RTX group had significantly lower BVAS at 6 months after treatment initiation.
compared with those with excessive B cell differentiation in the IV-CY group. BVAS in patients with excessive B cell differentiation in the RTX group were not significantly different from those in patients without excessive B cell differentiation, in either the RTX or IV-CY groups (Figs. 5A and Supplementary Fig. 2A).

Furthermore, patients with excessive B cell differentiation who achieved BVAS remission were compared. Although all patients receiving RTX achieved BVAS remission, none of those receiving IV-CY did. There were significantly more patients responding to treatment in the RTX group (RTX group = 56.3%, IV-CY group = 0.0%, p < 0.01) (Fig. 5B).

Next, in patients with excessive B cell differentiation, rates of GC dose reduction were compared. The RTX group showed higher rates of dose reduction at 3 months [RTX group = 67.6 ± 13.1%, IV-CY group = 47.2 ± 21.6%, p < 0.01] and 6 months [RTX group = 84.3 ± 7.0%, IV-CY group = 69.1 ± 21.9%, p = 0.03] of remission induction therapy than the IV-CY group (Supplementary Fig. 2B).

Regarding the incidence rates of adverse events in patients with excessive B cell differentiation, no significant difference was observed between the RTX and IV-CY groups. However, the incidence of severe infection was lower in the RTX group (RTX group = 4/16 patients, conventional therapy group

### Table 3

| Variables | Rituximab group | IV-CY group | p value |
|-----------|-----------------|-------------|---------|
| n (%)     |                 |             |         |
| Excessive b cell differentiation | 16 (24.1%) | 8 (14.8%) | 0.83 |
| Age (years) | 71.6 (8.3) | 69.4 (8.6) |         |
| Gender, n (%) | 8 (57.1%) | 4 (44.4%) | 0.67 |
| Disease duration (month) | 2 (2–4) | 3 (2–7) | 0.70 |
| new onset, n (%) | 16 (100.0%) | 8 (100.0%) | 1.00 |
| ANCA-positive at diagnosis, n (%) | 4 (25%) | 1 (12.5%) | 0.33 |
| Proteinase 3 ANCA | 3 (16.7%) | 0 (0.0%) |         |
| Myeloperoxidase-ANCA | 12 (75.0%) | 8 (100.0%) | 0.19 |
| ANCA-associated vasculitis type, n (%) | 8 (50.0%) | 11 (61.1%) | 0.35 |
| MPA | 11 (61.1%) | 7 (87.5%) |         |
| GPA | 7 (38.9%) | 1 (12.5%) |         |
| BVAS | 18.0 (5.6) | 14.5 (4.5) | 0.50 |
| GC dose at baseline | 54.6 (11.0) | 62.5 (22.4) | 0.51 |

Values listed as mean (SD) and median (minimum-maximum) unless otherwise stated. The statistical difference was determined by Bonferoni method, with chi-square test used for nominal variables. Difference with p < 0.05 was considered significant.
= 6/8 patients, \( p = 0.03 \), Fisher's exact test). Moreover, the 6-month survival rate after administration of high-dose GC therapy was significantly higher in the RTX group than in the IV-CY group (RTX group = 93.8%, IV-CY group = 62.5%, \( p = 0.0486 \)) (Fig. 5C).

**Discussion**

In the present study, which included patients with highly active AAV who were admitted to our hospital, we analyzed phenotypes of T and B cells before remission induction therapy to assess the clinical significance of these phenotypes and to differentiate the use of RTX and CY according to abnormal cell differentiation. Based on phenotyping of peripheral lymphocytes in this study, AAV patients exhibited a lower proportion of IgM memory B cells and a higher proportion of IgD−CD27− B cells. Thus, B cells were more differentiated in many patients with highly active AAV compared to those in HCs.

Regarding the association between peripheral B cells and clinical findings, it has been reported that peripheral CD5+ B cells are associated with relapse after remission induction therapy with RTX.[10, 11] However, because there has been no study comparing abnormal differentiation and clinical findings, it has remained unknown which lymphocyte phenotype is associated with prognosis. The present study revealed that B-cell phenotypes were not associated with disease activity or clinical findings but were correlated with responses to treatment. As the number of highly differentiated B cells increased, patients were more resistant to treatment. However, when patients with increased class-switched memory B cells or IgD−CD27− B cells, defined as those with excessive B cell differentiation, were compared, no differences were observed in either baseline patient characteristics or T cell phenotypes. The presence of abnormal B cell phenotypes could not be predicted from ANCA levels, other baseline patient characteristics, or disease activity. In contrast, many patients with excessive B cell differentiation were resistant to treatment so that detection of excessive B cell differentiation may be important for predicting prognosis.

The above indicates that changes in prognosis mainly based on excessive B cell differentiation may be useful for differentiating between the use of RTX, which is used for B-cell depletion therapy, and CY. The present study revealed that, in patients with excessive B cell differentiation, the concomitant
use of RTX was more effective than that of conventional therapy and yielded improvements similar to that achieved by patients without excessive B cell differentiation. In addition to showing that RTX is effective in patients who are resistant to CY or experience relapse after treatment with CY,[12-15] various clinical studies have demonstrated that RTX is as effective as CY against AAV accompanied by serious organ involvement.[3, 16] However, it is still unknown which patients should be treated with RTX and which with CY. The present study suggests that RTX is better indicated for patients with abnormal B cells. Moreover, this study also showed that the incidence of adverse events was lower in patients with excessive B cell differentiation who received a combination of RTX and GC than in those treated with concomitant conventional therapy, as GC doses could be reduced in the early stages. This may improve the survival rate in these patients.

Although the characteristics of B cells in AAV patients have been described in many reports, the importance of B cells remains controversial. Culton et al.[17] and Tadema et al.[18] reported that, compared with healthy individuals, AAV patients have more naive B cells (CD19+CD27−CD38−/low) and fewer memory B cells (CD19+CD27+CD38−/low). In contrast, Culton et al.[17] reported that, compared with healthy individuals, AAV patients have more CD19^{hi} memory B cells, which have an elevated capacity to produce autoantibodies. Moreover, Lepse et al.[19] reported that the proportion of regulatory B cells decreases in patients with highly active AAV. In the present study, phenotyping of peripheral lymphocytes revealed that AAV patients exhibited a low proportion of IgM memory B cells and a high proportion of IgD^{−}CD27^{−} B cells. Thus, in many patients with highly active AAV, B cells were more differentiated than in HCs, similar to a trend described in previous reports.[17, 18] The present study has several limitations. The RTX and IV-CY groups were not matched for baseline patient characteristics, and they were not compared in a double-blind manner. Thus, it cannot be ruled out that the selection of treatment was biased. However, no significant differences were observed between the RTX and IV-CY groups in terms of baseline patient characteristics, such as age, sex, disease duration, and disease activity, and the groups were compared for efficacy under the same conditions.
Conclusions
we performed 8-color flow cytometry in AAV patients before the administration of remission induction therapy and analyzed whether they exhibited excessive B cell differentiation. In patients with a high proportion of class-switched memory B cells or IgD−CD27− B cells, GC therapy combined with RTX is expected to be more effective than conventional therapy, allowing for early reduction of GC doses and yielding a higher survival rate. These results provide insight for the development of subclassifications of AAV based on analysis of immunophenotypes by flow cytometry and of corresponding therapeutic interventions in the form of precision medicine.

Abbreviations
AAV
Anti-neutrophil cytoplasmic autoantibody associated vasculitis
ANCA
Anti-neutrophil cytoplasmic autoantibody
BVAS
Birmingham Vasculitis Activity Score
CD
Cluster of differentiation
GC
Glucocorticoids
HC
Healthy controls
IV-CY
Intravenous cyclophosphamide pulse
RTX
Rituximab
SD
Standard deviation

Declarations
Ethics approval and consent to participate. This study was approved by the ethics review board of the University of Occupational and Environmental Health, Japan, and written informed consent was waived because of the retrospective study design.
**Consent for publication.** Not applicable.

**Availability of data and materials.** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing financial interests.** S. Nakayamada has received speaking fees from Bristol-Myers, Pfizer, Chugai, Sanofi, Astellas, Abbvie, Eisai, Asahi-kasei and has received research grants from Mitsubishi-Tanabe, Novartis and MSD. S. Kubo has received speaking fees from Bristol-Myers. Y. Tanaka has received consulting fees, speaking fees, and/or honoraria from Abbvie, Daiichi-Sankyo, Chugai, Takeda, Mitsubishi-Tanabe, Bristol-Myers, Astellas, Eisai, Janssen, Pfizer, Asahi-kasei, Eli Lilly, GlaxoSmithKline, UCB, Teijin, MSD, and Santen, and received research grants from Mitsubishi-Tanabe, Takeda, Chugai, Astellas, Eisai, Taisho-Toyama, Kyowa-Kirin, Abbvie, and Bristol-Myers. All other authors declare no conflict of interest.

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**Author contributions.** All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Miyazaki had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Figures
Figure 1

Changes in disease activity after 6 months (A) Changes in BVAS after 6 months of induction therapy by treatment group. Data are mean ± SD of BVAS in each treatment group. *p < 0.01 according to paired Student's t-test. (B) Rate of remission in BVAS by treatment group.

Statistical differences were determined by Fisher's exact test.

Figure 2

![Graph showing changes in naive B cell, IgM memory B cell, class switched memory B cell, and IgD-CD27- B cell percentages between HC and AAV.](image-url)
Figure 2

Proportions of peripheral T and B cell phenotypes in healthy control subjects and AAV patients at baseline. The statistical difference was determined by Student's t-test. Difference with p<0.05 was considered significant.

Figure 3
Correlations between clinical features and the proportion of peripheral naive B cells or class-switched B cells at baseline. Correlations between disease activity at baseline and the proportion of peripheral naive B cells or class-switched B cells at baseline (top). Correlations
between the rate of improvement in BVAS and the proportion of peripheral naive B cells or class-switched B cells at baseline (bottom). The correlation between immune cell phenotypes and clinical features was analyzed by the Pearson product-moment correlation coefficient. The level of significance was considered p<0.05. r: correlation coefficient.

Figure 4

A

Healthy Control

Switch memory B cells
15.5%

IgD- CD27- B cells
5.06%

Patients with ANCA-associated vasculitis

Switch memory B cells
12.2%

Switch memory B cells
62.9%

Switch memory B cells
51%

IgD- CD27- B cells
23.7%

B

Excessive B cell differentiation (+)
24/54 (44.4%)

Excessive B cell differentiation (-)
30/54 (55.6%)

C

Excessive B cell differentiation (+)
16.8 ± 5.5

Excessive B cell differentiation (-)
16.3 ± 6.8

D

Rate of BVAS remission

37.5

66.7

*
Identification and proportion of excessive B cell differentiation (A) Human peripheral blood CD19+CD20+ B cells were stained with CD27 and IgD and separated into four phenotypes: CD27–IgD–, IgM memory (CD27+IgD+), class-switched memory (CD27+IgD–), and naive (CD27–IgD+) B cells, as indicated by the four quadrants. Dot plots show representative data from healthy control subjects (n = 15, top), AAV patients without excessive B cell differentiation (bottom left), and AAV patients with excessive B cell differentiation (bottom middle and right). (B) Proportions of AAV patients with (grey) and without (black) excessive B cell differentiation. (C) Changes in BVAS after 6 months of induction therapy in patients with and without excessive B cell differentiation. Data are mean ± SD of BVAS in each group. *p < 0.01 according to paired Student’s t-test. (D) Rates of remission in BVAS in patients with and without excessive B cell differentiation. Data show the median rate of improvement in BVAS in each group. *p < 0.01 according to Fisher’s exact test.
Figure 5
RTX was effective to conventional immunosuppressants in patients with excessive B cell differentiation. (A) Rate of improvement in BVAS after 6 months of induction therapy in patients with and without excessive B cell differentiation by treatment group. Data show the median rate of improvement in BVAS. **p<0.05, *p < 0.01 according to Bonferroni method. (B) Rate of remission after 6 months of induction therapy in patients with excessive B cell differentiation by treatment group. *p < 0.01 according to Fisher’s exact test. (C) Differences between RTX and IV-CY groups in survival rates after 6 months of induction therapy in patients with excessive B cell differentiation.

Supplementary Files
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AAV bcell (AR&T) Supplementary_materials.docx