Altered B cell balance, but unaffected B cell capacity to limit monocyte activation in anti-neutrophil cytoplasmic antibody-associated vasculitis in remission

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Abstract

Objective. Regulatory B cells (Bregs) constitute a subset of B cells with immunomodulatory properties. Numerical and functional alterations in the Breg compartment have been associated with autoimmunity. The aim of this study was to assess the frequency and function of Bregs in patients with ANCA-associated vasculitis (AAV).

Methods. B cell subsets were determined in the peripheral blood of 48 AAV patients (12 active, 36 in remission) and 41 healthy controls (HCs) by flow cytometry. Bregs were defined within the CD19+ population as CD24hiCD38hi or CD24hiCD27+ cells. The percentage of IL-10-positive B cells in circulation was analysed by flow cytometry. Sorted CD19+ B cells were co-cultured with monocytes to evaluate their capacity to inhibit monocyte TNF-α production upon lipopolysaccharide stimulation.

Results. The frequency of circulating CD19+CD24hiCD38hi cells was not different in AAV patients in remission compared with HCs, but was decreased in patients with active disease [mean in HCs 5.5% (s.d. 1.6) vs active 3.8% (s.d. 2.8), P = 0.0104]. Furthermore, the percentage of CD19+CD24hiCD27+ cells was significantly decreased in both remission and active patients when compared with HCs [HCs 15.0% (s.d. 9.3) vs remission 6.6% (s.d. 4.4) (P < 0.0001) vs active 6.4% (s.d. 6.2) (P = 0.0006)]. The frequency of IL-10-positive B cells was comparable between patients and HCs. B cells from AAV patients suppressed monocyte TNF-α production to a similar extent to cells from HCs.

Conclusion. Based on immunophenotypic classification, Bregs are numerically diminished in AAV patients. However, B cell function in terms of IL-10 production and their capacity to suppress monocyte activation is not compromised in AAV patients in remission.

Key words: ANCA-associated vasculitis, B cells, regulatory B cells, IL-10.

Introduction

ANCA-associated vasculitides (AAVs) comprise a group of severe inflammatory diseases characterized by the presence of autoantibodies that target specific neutrophil cytoplasmic proteins, in particular PR3 and MPO [1]. AAVs primarily affect small- to medium-sized blood vessels, and based on clinical and pathological features, three disease entities can be distinguished—granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic GPA [2, 3].

Recently two major clinical trials demonstrated that treatment with the B cell–depleting antibody rituximab is efficacious in inducing disease remission in AAV [4, 5], and continuous B cell depletion might be a useful strategy for remission maintenance [6, 7]. These results underscore the crucial role of B cells in AAV pathogenesis. However, the underlying mechanisms are complex and
Under physiological circumstances, B cells are critical players in the regulation of immune responses, providing protection against infection without causing overt damage to the host. This is accomplished through a finely regulated balance between B cell effector and regulatory functions exerted in an antibody-dependent as well as an antibody-independent fashion [8]. An increasing body of evidence indicates that in autoimmunity the interplay between pathogenic and protective B cell functions is dysregulated. In this context, the identification of regulatory B cells (Bregs) in both mice and humans has gained considerable interest in the autoimmunity field. Bregs are defined by their capacity to suppress immune responses primarily via provision of IL-10 [9]. A number of studies in mouse models of autoimmunity have shown that IL-10-producing B cells are capable of suppressing disease development [10–13]. IL-10-producing Bregs and their possible contribution to autoimmune responses have also been studied in humans. Identification of circulating Bregs in humans has been suggested based on high expression of CD24 in combination with expression of either CD38 [14] or CD27 [15] (CD19+CD24hiCD38hi or CD19+CD24hiCD27+). Interestingly, dysregulated frequencies and/or defective function of Bregs have been documented in autoimmune diseases, such as multiple sclerosis (MS) [16, 17], SLE [14] and RA [18]. CD19+CD24hiCD38hi cells from normal individuals were shown to suppress IFN-γ production by CD4+ T cells, while in SLE patients B cell suppressive function was reduced [14]. Recent data also indicate that CD19+CD24hiCD38hi cells inhibit the differentiation of naive T cells into Th1 or Th17 cells and promote the development of regulatory T cells [18]. These effects were mediated in part by provision of IL-10 and were found to be partially impaired in RA patients. Others have reported that IL-10-producing Bregs reside in the CD19+CD24hiCD27+ subset, which can suppress TNF-α production by monocytes [15].

Overall, current evidence indicates that Bregs play an important immune regulatory role and suggests that alterations in either number or function of Bregs contribute to pathogenic immune responses in autoimmune diseases. However, so far data on Breg frequency and/or function in AAV patients is limited. In the present study the frequencies of Bregs in peripheral blood were assessed in a cohort of AAV patients and healthy individuals based on cell surface markers. In addition, the capacity of peripheral B cells to produce IL-10 and their ability to suppress monocyte TNF-α production were determined.

Patients and methods

Study population

Peripheral blood was collected from 48 PR3-AAV patients [mean age 57.8 years (s.d. 13.6)] and 41 age- and sex-matched healthy controls [HCs; mean age 54.5 years (s.d. 7.4)]. The characteristics of patients and HCs included in the study are described in Table 1. Thirty-two of 48 patients (67%) received oral CYC (2 mg/kg/day) and prednisolone (60 mg/day) for induction therapy. None of the patients had received rituximab prior to inclusion in this study. Patients were at least 3 months without CYC treatment before inclusion in this study. The diagnosis of GPA or MPA was based on the definitions outlined in the Chapel Hill Consensus Conference [19], and GPA patients fulfilled the classification criteria of the ACR [19, 20]. Samples were obtained in compliance with the Declaration of Helsinki. All subjects provided informed consent and the study was approved by the Medical Ethics Committee of the University Medical Center Groningen, University of Groningen.

Flow cytometry for B cell phenotype analysis

The expression of CD24, CD27 and CD38 by peripheral blood B lymphocytes was determined by flow cytometry. Briefly, freshly drawn EDTA blood was washed twice with PBS supplemented with 1% BSA to remove the plasma. After washing, cells were suspended in PBS plus 1% BSA to the original volume and 100 μl of the cell suspension were incubated with anti-human CD19-eFluor-450 (clone HIB19; eBioscience, San Diego, CA, USA), anti-human CD24-FTC (clone ML5; BD Biosciences, San Jose, CA, USA), anti-human CD27-APC-eFluor-780 (clone O323; eBioscience), anti-human CD38-PE-Cy7 (clone HIT2; eBioscience) or the corresponding isotype control antibodies for 15 min in the dark. The red blood cells were lysed with FACS Lysing Solution (BD Biosciences). Cells were washed with PBS plus 1% BSA and analysed with an LSR-II flow cytometer (BD, Franklin Lakes, NJ, USA). The data were analysed using Kaluza 1.2 flow analysis software (Beckman Coulter, Brea, CA, USA).

Detection of IL-10-producing B cells by flow cytometry

According to the previously described methodology [15], IL-10-producing B cells were quantified using two approaches. Based on the method used, the IL-10-positive B cells are termed either B10 or B10pro cells. For detection of B10 cells, heparinized blood was diluted 1:1 with RPMI 1640 (Lonza, Basel, Switzerland) and stimulated for 4 h at 37 °C with 50 ng/ml of phorbol myristate acetate (PMA; Sigma-Aldrich, St Louis, MO, USA) and 2 mM calcium ionophore (Sigma-Aldrich) in the presence of 10 μg/ml brefeldin A (BFA; Sigma-Aldrich). At the end of the culture the red blood cells were lysed with ammonium chloride buffer and the remaining cells were washed with PBS plus 5% fetal calf serum (FCS; Lonza). The cells were stained with anti-human CD19-eFluor-450 and anti-human CD22-PE-Cy5 (clone HIB22; BD Biosciences) for 15 min in the dark. The cells were then fixed and permeabilized using the Fix&Perm kit (Invitrogen, Life Technologies, Grand Island, NY, USA) and incubated with anti-human IL-10-PE (clone JES3-9D7; BioLegend, San Diego, CA, USA) to stain for intracellular IL-10 expression.

For induction of B10pro cells, peripheral blood mononuclear cells (PBMCs) were cultured in RPMI 1640 (with 50 μg/ml gentamicin (GIBCO, Life Technologies)
TABLE 1 Characteristics of the study population

<table>
<thead>
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<th>Characteristics</th>
<th>HCs</th>
<th>AAV remission</th>
<th>AAV active</th>
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<tr>
<td>Number of subjects, n (males, %)</td>
<td>41 (59)</td>
<td>36 (61)</td>
<td>12 (58)</td>
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<td>57.8 (27–81)</td>
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</tr>
<tr>
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<td>Frequency of clinical manifestations, n (%)</td>
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<td>CYC, pred, plasma exchange</td>
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<td>BVAS, median (range)</td>
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AAV: ANCA-associated vasculitis; GPA: granulomatosis with polyangiitis; HCs: healthy controls; MP: methylprednisolone; MPA: microscopic polyangiitis.

and 10% FCS] at a concentration of $1 \times 10^6$ cells/mL. Cells were left untreated or were stimulated with CpG-ODN-2006 (500 ng/mL; Hycult Biotech, Uden, the Netherlands) for 72 h. In the last 5 h the cells were re-stimulated with PMA (50 ng/mL) and calcium ionophore (2 mM) in the presence of BFA (10 μg/mL). Cells were washed with PBS plus 5% FCS and stained using the same protocol as described above for B10 cells. All samples were analysed with an LSR-II flow cytometer and the data were processed using Kaluza 1.2 flow analysis software.

Monocyte suppression assay

B cell suppressive capacity was evaluated as described before [15]. PBMCs were isolated from heparinized blood and cells were labelled with anti-human CD19-Fluor-450. CD19+ cells were sorted with fluorescence-activated cell sorter (FACS; MoFlo, Beckman Coulter); based on the post-sort purity analysis, the obtained population was >95% pure. Sorted B cells were cultured in the presence or absence of CpG-ODN 2006 (1 μg/mL) and anti-CD40 (1 μg/mL; clone 5C3; eBioscience) for 24 h. Monocytes were isolated using the IMag Monocyte Enrichment Set (BD Biosciences) according to the manufacturer’s instructions. After 24 h, B cells were washed and monocytes were added in a ratio of 1:1. B cells and monocytes were co-cultured for 24 h. In the last 4 h, 1 μg/mL lipopolysaccharide (LPS; Ultra pure; InvivoGen, San Diego, CA, USA) and BFA (10 μg/mL) were added to induce TNF-α production in monocytes. At the end of the experiment, cells were washed with PBS plus 5% FCS and stained with anti-human CD14-PerCP-Cy5.5 (clone M5E2; BD Biosciences; clone MAb11; BD Biosciences). Samples were analysed with FACSCalibur (BD Biosciences) and Kaluza 1.2 flow analysis software was used for data analysis.
Statistical analysis

Data represent median values unless stated otherwise. For comparison of more than two groups, one-way analysis of variance was used if the data were normally distributed and Kruskal-Wallis test was applied if the data had a non-Gaussian distribution, as determined by the D’Agostino and Pearson omnibus normality test. If a significant difference was found, further testing was done using an unpaired t test or Mann-Whitney test for data with Gaussian and non-Gaussian distribution, respectively. A P-value <0.05 was considered statistically significant.

Results

Altered distribution of B cell subsets in AAV patients

Phenotypic characterization of the circulating B cell population was performed in 48 AAV patients and 41 age- and gender-matched HCs. Lymphocytes were gated from the forward-side scatter based on their size and expression of CD19 was used to separate B cells (Fig. 1A). Transitional, naive and memory B cells were characterized based on expression of CD27 and CD38 (Fig. 1B). Surface expression of CD24 in combination with CD27 or CD38 was used to distinguish two proposed Breg subsets (Fig. 1C and D).

The percentage of CD19+ B cells within the circulating lymphocyte population did not differ between AAV patients and HCs (Fig. 2A). AAV patients with active disease had a decreased percentage of transitional B cells when compared with HCs or patients in remission (Fig. 2B). Additionally, AAV patients had an increased percentage of naive B cells independent of disease activity (Fig. 2C). In line with previous studies [21], we found a significantly decreased proportion of circulating memory B cells in AAV patients when compared with HCs (Fig. 2D).

Further, we characterized the CD19+CD24highCD38high and CD19+CD24highCD27+ subsets, which have been reported to have immunoregulatory properties in humans.

Fig. 1 Gating strategies for B cell subsets

Representative flow cytometry dot plots from a healthy individual. Lymphocytes were gated based on the forward-side scatter profile. (A) Within the lymphocyte population, B cells were distinguished by expression of CD19. (B) Cell surface markers CD27 and CD38 were used to further characterize B cell subpopulations. Transitional B cells were CD19+CD27-CD38high, naive B cells were defined as CD19+CD27+CD38low and memory B cells were CD19+CD27+CD38low. The two putative regulatory B cell subsets were defined as (C) CD19+CD24highCD38high or (D) CD19+CD24highCD27+. 
Interestingly, the CD19^+CD24^{high}CD38^{high} population was overlapping with the transitional B cell subset, whereas CD19^+CD24^{high}CD27^- cells represented a subset of the memory B cell population (Fig. 1B-D). Thus the proposed regulatory subsets represent two distinct populations. The proportion of CD19^+CD24^{high}CD38^{high} cells was not different between HCs and AAV patients in remission, but was significantly decreased in patients with active disease when compared with HCs and patients in remission (Fig. 2E). Also, the percentage of CD19^+CD24^{high}CD27^- cells was diminished in both remission and active patients when compared with HCs (Fig. 2F).

Effect of treatment on B cell subset distribution

In order to rule out the effect of treatment on B cell subset distribution, we compared the percentage of total B cells and B cell subsets in untreated (n = 34) and treated (n = 14) patient groups. When patients were subdivided based on treatment but independent of disease activity, no significant differences were found in the percentage of circulating total B cells or any of the given B cell subsets. When only patients in clinical remission (n = 36) were subdivided based on treatment, treated patients (n = 10) had a significantly decreased percentage of circulating B cells, and none of the B cells subsets were significantly affected by the treatment (Table 2). Additionally, we analysed the possible relationship between clinical phenotypes and the proposed Breg subsets. The percentage of circulating CD19^+CD24^{high}CD38^{high} or CD19^+CD24^{high}CD27^- cells was not associated with renal, ENT, chest involvement or the frequency of relapses.
The percentage of IL-10-producing B cells is not diminished in AAV patients

Previous studies have suggested the existence of a rare B cell subset (termed B10 cells) that represents ~1% of circulating B cells and is capable of producing IL-10 upon short stimulation with PMA and calcium ionophore. Additionally, a subset of peripheral blood B cells can be induced in vitro to become IL-10-producing B cells (B10pro cells) after prolonged stimulation with Toll-like receptor (TLR) agonists [15]. As IL-10 is considered the signature effector cytokine of Bregs, we evaluated the frequency of both naturally occurring IL-10-competent B cells (B10 cells) and inducible IL-10-producing B cells (B10pro cells) in order to indirectly examine the functionality of Bregs in AAV patients.

For quantification of the B10 cells, whole blood was stimulated for 4 h with PMA and calcium ionophore (Fig. 3A). Stimulation in this manner induced IL-10 expression in 0.82% (range 0.41–2.03) of B cells in samples from HCs (n = 10). A similar level of B10 cells was found in AAV patients (n = 10), who had 0.82% (range 0.19–1.96) of IL-10-positive B cells (Fig. 3B).

To determine the frequency of the B10pro cells, PBMCs were stimulated with TLR9 ligand CpG-ODN for 3 days (Fig. 3C). The frequency of the inducible IL-10-positive B cells was measured in 18 HCs, 15 remission patients and 6 active patients (Fig. 3D). In the HC group, the median percentage of IL-10-positive B cells was 9.2% (range 3.0–19.9), and this was not significantly different from the median level found in AAV patients in remission (median 8.1%, range 2.5–18.0) or patients with active disease (10.4%, range 4.9–21.8) (Fig. 3D). These data suggest that the overall capacity of B cells to produce IL-10 is not compromised in AAV patients.

B cell capacity to suppress monocyte activation is comparable in AAV patients and HCs

To investigate whether B cells from AAV patients can suppress pro-inflammatory cytokine production by other immune cells, we tested their ability to inhibit TNF-α production by monocytes activated with LPS. As patients often present with low numbers of lymphocytes, total CD19⁺ B cells were used in this suppression assay.

Sorted CD19⁺ cells were activated with CpG-ODN and stimulatory anti-CD40 antibody and then co-cultured with autologous monocytes in a ratio of 1:1 for 24 h. In the final 4 h of the culture LPS was added to induce TNF-α production in monocytes. LPS-activated monocytes cultured in the absence of B cells were used as a positive control. For each individual, TNF-α expression in the positive control sample was set at 100% and the B cell-mediated suppression was calculated accordingly (Fig. 4A).

When monocytes were cultured in the presence of B cells that were not activated with CpG-ODN and anti-CD40, the median TNF-α production in monocytes was reduced by 38% in HCs and by 46.5% in GPA patients (Fig. 4B). This B cell-mediated suppressive effect was further enhanced if B cells had been activated with CpG-ODN and anti-CD40 prior to co-culture with monocytes, resulting in a median inhibition of 60% in HCs and 58% suppression in patients (Fig. 4B). These results demonstrate that B cells from AAV patients can suppress monocyte activation to a similar extent as those obtained from HCs, indicating that in AAV patients in remission B cell inhibitory function is not compromised.

### Discussion

AAVs are autoimmune disorders in which both innate and adaptive immune mechanisms contribute to disease pathogenesis. Breg cells have been shown to influence both arms of the immune system and have the potential to exert their modulatory effects on immune cells involved in the pathogenesis of AAV. In this study we characterized circulating B cell subpopulations, including two putative Breg subsets, in patients with AAV and age- and sex-matched HCs. AAV patients during active disease had decreased frequencies of CD19⁺CD24⁺CD38high cells, while the proportions of CD19⁺CD24⁺CD27⁺ cells were diminished independent of disease activity. However, no differences were found in the percentages of IL-10-producing B cells between AAV patients and HCs. In addition, B cells from AAV patients in remission inhibited monocyte activation to a similar extent as B cells obtained from HCs.

The data presented in this study show an overall altered distribution of transitional, naive and memory B cell subsets in AAV patients. In line with previous studies, we
found a diminished frequency of memory B cells [21] independent of disease activity. In patients with active disease, the frequency of transitional B cells was also diminished when compared with HCs or patients in remission. Interestingly, the two putative Breg subsets CD19+CD24highCD38high and CD19+CD24highCD27+ were not overlapping and were contained within the transitional [22] and memory B cell compartments, respectively. Therefore the reduced proportion of circulating CD19+CD24highCD38high and CD19+CD24highCD27+ cells that was observed in patients is probably due to diminished transitional and memory subsets accordingly. Currently the reason for changes in B cell subset distribution is unclear. However, since B cells are known to be present in granulomatous lesions of AAV patients [23], we cannot exclude the possibility that the observed alterations in the peripheral blood occur due to B cell migration to the site of inflammation.

In humans, no consensus has been reached yet with regard to cell surface markers that identify a unique Breg population and none of the currently proposed markers can exclusively distinguish IL-10-producing B cells from other B cell subsets. Also, a specific transcription factor, which could potentially be used to separate IL-10-producing B cells, has not been identified. Moreover, although studies have proposed the T cell Ig domain and mucin domain protein 1 (TIM-1) as a useful marker for delineation of Bregs in mice [24], preliminary experiments from our group could not confirm this in humans (see supplementary Fig. S1, available at Rheumatology Online). Recently van de Veen et al. [25] used a whole-genome expression array to characterize markers differentially expressed by human IL-10-producing B cells compared with B cells that do not produce IL-10. Surprisingly, none of the previously reported markers was found to be specific for the IL-10-positive B cell compartment. The frequency

![Fig. 3](https://example.com/figure3.png)

**Fig. 3** The percentage of IL-10-producing B cells in AAV patients and HCs. (A and B) For detection of B10 cells, whole blood samples were stimulated with PMA and calcium ionophore in the presence of BFA for 4 h. (C and D) For induction of B10pro cells, PBMCs were stimulated with CpG-ODN for 3 days. PMA, calcium ionophore and BFA were added in the final 5 h of the culture. (A and C) Representative flow cytometry dot plots from a healthy individual, demonstrating the gating strategy for (A) B10 cells and (C) B10pro cells. Samples incubated with BFA only were used to set the gate. (B) Frequency of B10 cells from HCs (n = 10) and AAV patients (n = 10) in remission. (D) Percentages of B10pro cells in HCs (n = 18), AAV patients in remission (n = 15) and AAV patients with active disease at the time of inclusion (n = 6). PMA: phorbol myristate acetate; BFA: brefeldin A; HCs: healthy controls; AAV: ANCA-associated vasculitis.
of IL-10-positive B cells was found to be similar in CD19+CD24highCD38high, CD19+CD24intCD38int and CD19+CD24highCD38−/C0 subsets. Also the percentage of CD27+ B cells was equal between IL-10-positive and IL-10-negative B cells. This could explain why in our study the patients with decreased proportions of CD19+CD24highCD38high or CD19+CD24highCD27− cells still displayed a normal frequency of IL-10-producing B cells. In contrast to our observations, a recent report by Wilde et al. [26] demonstrated a significantly decreased proportion of IL-10-positive B cells in the circulation of AAV patients. Additionally, in untreated patients in clinical remission, a negative association was found between IL-10-positive B cells and Th1 cells, which is in line with previous studies that suggested Breg mediated suppression of the Th1 lineage [14, 18]. At present we cannot explain these discrepancies.

However, it should be taken into account that although IL-10-expressing B cells are considered the gold standard for distinguishing the Breg subset, Bregs also function through IL-10-independent mechanisms. These include production of TGF-β [27] as well as interaction with target cells via molecules expressed on the cell surface, such as CD80 and CD86 [14]. Our data also strongly suggest that B cells to a large extent suppress monocyte activation in an IL-10-independent manner, as monocyte co-culture with B cells without CpG-ODN and anti-CD40 activation resulted in significant inhibition of TNF-α production in monocytes. As B cells do not produce IL-10 without prior activation, this suggests that B cells inhibit TNF-α production through an alternative mechanism, although we have not dissected whether this inhibition is mediated via production of another soluble factor or through a cell–cell interaction.

In AAV, autoantibodies are considered central players in disease pathogenesis, as ANCA-mediated neutrophil and monocyte activation is considered a critical event leading to blood vessel wall injury [1]. The use of B cell depletion therapy in AAV patients has not only proven clinically successful, but also has given more insight into the role of B cells in AAV. Rituximab-treated patients achieved clinical remission before the decline of circulating autoantibodies, suggesting that B cells also exert pathogenic effects in an autoantibody-independent fashion [5], although the underlying mechanisms are not yet clear. Also, patients benefit from B cell depletion therapy even when Bregs are being depleted, suggesting that B cell-mediated pro-inflammatory effects dominate over their regulatory function. Recent studies have identified IL-6 as an important candidate effector cytokine that mediates the pro-inflammatory effects of B cells [28]. In the experimental autoimmune encephalomyelitis model, B cells are a major source of IL-6, and mice lacking IL-6-producing B cells had reduced disease severity associated with reduced IL-17 production by CD4+ T cells. Also, B cells isolated from MS patients secreted elevated amounts of IL-6 when compared with HCs. Interestingly, IL-6 production by B cells was normalized in the reconstituting B cell population after B cell depletion therapy. This suggests that the beneficial effects of B cell depletion therapy could be due in part to elimination of IL-6-producing B cells, which in turn leads to diminished Th17 responses. Since disturbances in T helper cell subsets

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**Fig. 4** B cell-mediated suppression of TNF-α production in monocytes

In five HCs and five AAV patients in remission, B cell suppressive capacity was tested using LPS-activated monocytes as a responder population. Intracellular TNF-α expression in monocytes was measured by flow cytometry. **(A)** Representative histograms showing TNF-α expression in monocytes. Monocytes cultured alone without adding LPS were used as a negative control. Monocytes activated with LPS served as a positive control sample. Monocytes were co-cultured with either unstimulated B cells or with B cells that were preactivated with CpG-ODN and anti-CD40. **(B)** Data from five HCs and five GPA patients. For each donor the median FI of TNF-α expression in the positive control condition was used as a reference sample and was normalized to 100%. Median FI values measured in other samples are expressed relative to the reference sample. LPS: lipopolysaccharide; HCs: healthy controls; GPA: granulomatosis with polyangiitis; FI: fluorescence intensity.
[29–31], including increased Th17 cells [32–34], are well documented in AAV patients, future studies should determine not only the changes in the newly reconstituted B cell populations, but also the effects of B cell depletion therapy on the T cell compartment.

The (im)balance between regulatory and effector functions of B cells could contribute to the pathogenesis of autoimmune diseases, however, in AAV data on regulatory and effector B cells are limited. A recent study by Bunch et al. [35] reported that the percentage of circulating CD5⁺ B cells, which partially overlap the CD19⁺CD24highCD38high phenotype, could be used as a biomarker to monitor disease activity and risk for relapse. A low percentage of circulating CD5⁺ B cells was shown to correlate with disease activity and shorter time to disease relapse. Also, patients after rituximab treatment whose B cells repopulated with a low percentage of CD5⁺ B cells in conjunction with no or low immunosuppressive therapy were prone to relapse earlier than those who received high levels of maintenance immunosuppression. Although follow-up data were available from a limited number of patients, these data suggest that monitoring the B cell compartment could be informative in determining disease progression and activity, and possibly aid in predicting disease relapse.

The current study was designed as a cross-sectional study, and one of the main limitations is the lack of longitudinal data. Therefore the current data set cannot provide information on alterations that possibly occur in the B cell compartment within individual patients during the course of the disease. Also, the number of patients that presented with active disease during this study (n = 12) is limited and the consistency of findings in active disease should be verified in a larger cohort of patients. Although we confirmed that during remission the B cell suppressive capacity was not disturbed, further investigations are needed to determine whether this is also the case during active disease.

In summary, the putative Breg subsets that have been proposed to harbour IL-10-competent B cells were diminished in the circulation of AAV patients. However, the ability of B cells to produce IL-10 and their capacity to suppress activation of other immune cells were not compromised in patients in clinical remission, implying that Bregs are functional in AAV patients with quiescent disease. Longitudinal studies in remitting–relapsing disease are needed to evaluate whether Bregs contribute to sustaining stable remission in AAV.

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Supplementary data

Supplementary data are available at Rheumatology Online.

References


Rheumatology key messages

- ANCA-associated vasculitis (AAV) patients have dysregulated homeostasis of circulating B cells.
- B cell capacity to produce IL-10 and suppress monocyte activation is not impaired in AAV patients in remission.
- Future studies should investigate the clinical relevance of (im)balance between B cell pathogenic and suppressive functions in AAV.


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