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Mycophenolic Acid and 6-Mercaptopurine both Inhibit B cell Proliferation in Granulomatosis with Polyangiitis Patients, whereas Only Mycophenolic Acid Inhibits B cell IL-6 Production

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Manuscript in preparation
Abstract

Background Granulomatosis with polyangiitis (GPA) is a relapsing autoimmune disease affecting small- to medium-sized blood vessels. B cells are important in the pathogenesis of GPA as precursors of anti-neutrophil cytoplasmic autoantibody producing cells and likely also contribute to disease activity via antibody (Ab)-independent effects. Mycophenolate mofetil (MMF) and azathioprine (AZA) are immunosuppressive therapies used to maintain remission in these patients. Although, MMF-treated GPA patients are more prone to relapses than AZA-treated patients, the underlying mechanisms and effects on B cells are unknown. The objectives of this study were to investigate whether treatment with MMF and AZA alters the circulating B cell balance and whether the active compounds of these drugs have a differential effect on in vitro B cell proliferation and cytokine production in GPA patients and HCs.

Methods Circulating B cell subset frequencies were determined by flow cytometry in samples from 13 AZA-treated, 13 MMF-treated, 19 untreated GPA patients and 41 matched healthy controls (HCs). To determine the ex vivo effect of the active compounds of MMF and AZA, MPA and 6-MP, respectively, on B cell proliferation and cytokine production, peripheral blood mononuclear cells of 29 untreated GPA patients and 30 matched HCs were cultured for 3 days in the presence of CpG with 6-MP or MPA. After restimulation with PMA and Ca-I, cytokine-positive B cell frequencies were determined. To determine the effect of MMF and AZA treatment on in vitro B cell proliferation and cytokine production, PBMCs of 18 MMF-treated, 28 AZA-treated and 41 HCs were isolated and cultured in the presence of CpG.

Results Decreased transitional B cell and CD24hiCD38hi Breg frequencies were detected in AZA-treated patients, whereas in MMF-treated patients memory B cell frequencies were lower compared to HCs. Decreased CD24hiCD27+ Bregs were found in AZA- and MMF-treated patients and untreated patients compared to HCs. MPA and 6-MP addition to CpG-stimulated PBMC cultures significantly decreased B cell proliferation, but MPA was in HCs more effective than 6-MP. In vitro B cell proliferation in MMF- and AZA-treated patients was not inhibited. B cell IL-6 production was decreased in vitro by MPA compared to addition of 6-MP and CpG only. No effect was seen on the IL-10* or TNFα* B cell frequencies in GPA patients. Also, no differences in IL-10+, IL-6+ or TNFα+ B cell proportions or B cell proliferation were found in patients actively treated with MMF or AZA.

Conclusions While GPA patients treated with MMF show an increased relapse rate, our results indicate that MMF is superior to AZA in inhibiting B cell cytokine production in GPA patients. Future studies should assess the effects of these immunosuppressive drugs on other immune cells to elucidate mechanisms underlying the differences in relapse rates.
Introduction

Granulomatosis with polyangiitis (GPA) is a systemic autoimmune disease characterized by inflammation of small- to medium-sized blood vessels. GPA is associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA) mainly directed against proteinase 3. Patients with GPA frequently suffer from severe disease relapses that increase the disease burden.

Patients suffering from GPA and other autoimmune diseases such as systemic lupus erythematosus (SLE) receive induction- and maintenance immunosuppressive therapy to treat active disease and prevent disease relapses, respectively. Remission maintenance therapy often consists of azathioprine (AZA) or mycophenolate mofetil (MMF) combined with glucocorticoids. The active compounds of both AZA and MMF inhibit purine nucleotide synthesis, which is important for DNA synthesis and lymphocyte proliferation. The active compound of MMF, mycophenolic acid (MPA), inhibits the enzyme inosine monophosphate dehydrogenase 2 (IMPDH2), an isotype which is specifically upregulated in activated lymphocytes. The active compound of AZA, 6-mercaptopurine (6-MP), non-selectively inhibits IMPDH resulting in inhibition of all activated immune cells.

B cells play an important role in the GPA pathogenesis as precursors of ANCA-producing plasma cells. Importantly, B cells also exert antibody (Ab)-independent properties such as antigen presentation and cytokine production. These Ab-independent B cell functions in GPA gained more interest since rituximab, a CD20+ B cell depleting monoclonal Ab, was proven effective in inducing and maintaining disease remission. Although the ANCA-producing CD20 plasma cells are not targeted by rituximab, a gradual decrease in serum ANCA is seen upon B cell depletion by rituximab and induction of remission in GPA patients. This finding indicates that the sole presence of ANCA in the circulation, in the absence of CD20+ B cells, does not induce GPA reactivation and underlines the importance of Ab-independent functions of B cells in the GPA pathogenesis. Indeed, we previously demonstrated several alterations in the B cell compartment of GPA patients. An altered B cell subset balance in GPA patients was found, characterized by increased circulating naive and decreased memory B cell and regulatory B cell (Breg) frequencies. Moreover, Bregs of GPA patients correlated inversely with T helper (Th) 17 cells and showed decreased suppression of IL-17-producing Th cells compared to HCs. Lastly, we recently reported that B cells of GPA patients show increased sensitivity of the B cell receptor signaling pathway compared to HCs, possibly making GPA B cells more prone to become activated.

Although both AZA and MMF are used to maintain disease remission, limited studies are available comparing the effectiveness of both drugs. The only controlled study in ANCA-associated vasculitis (AAV) patients comparing these drugs showed MMF to be less effective in maintaining disease remission compared to AZA. This contrasts with...
treatment outcome in SLE patients in whom MMF is similar or even more effective in remission maintenance compared to AZA. In SLE, MMF treatment resulted in a decreased proportion of antigen-experienced B cells (i.e. memory B cells, plasmablasts and plasma cells), reduced serum IgG and diminished in vitro B cell IgG and IgM production. In vitro stimulation of B cells with MPA resulted in decreased B- and plasma cell formation. Moreover, in vitro B cell IL-10 production was dose-dependently inhibited by MPA. Together these findings point to a predominant effect of MMF on the Ab-producing function of B cells in SLE. The humoral function of B cells is well known to be an important driver of the SLE pathogenesis, whereas this is less clear in GPA. Importantly, little is known about the in vitro effects of 6-MP and MPA on B cells of GPA patients. Moreover, it is not known how differences herein possibly relate to (future) disease relapses in GPA.

The current study aimed to assess the in vivo and in vitro effects of AZA and MMF on B cells of GPA patients. Also, we aimed to relate our findings to the previously reported differences in effectiveness of AZA and MMF to maintain remission in GPA patients. We hypothesized that the difference in relapse rates between MMF- and AZA-treated GPA patients may be due to drug-related differential effects on B cell subtype distribution and/or function.

Materials and Methods

Study Population

In total, 75 currently or historically PR3-ANCA positive GPA patients in stable disease remission with or without maintenance immunosuppressive therapy for at least six months were enrolled in this study. The diagnosis of GPA was based on criteria determined in the Chapel Hill Consensus Conference and classification criteria of the American College of Rheumatology were met. Remission was defined as absence of clinical disease activity as reflected by a Birmingham vasculitis activity score (BVAS) score of zero. All patients had received induction therapy with cyclophosphamide and corticosteroids.

To determine changes in B cell phenotype between treatment groups and in vitro B cell cytokine production, 13 AZA-treated GPA patients, 13 MMF-treated GPA patients and 22 age-matched healthy controls (HCs) were included (Treated Cohort 1; Table 1). To assess the in vitro effect of the active compounds of AZA and MMF (6-MP and MPA, respectively) on these parameters, we included 19 GPA patients in remission, receiving no (n=18) or only a low dose prednisolone (n=1) and 19 age- and sex-matched HCs (Untreated Cohort 1; Table 1). To study the in vitro effect of 6-MP and MPA on B cell proliferation, we included 10 remission GPA patients receiving no immunosuppressive medication and 11 matched HCs (Untreated Cohort 2; Table 1). To determine in vitro
B cell proliferation in treated patients, we included 15 AZA-treated GPA patients, 5 MMF-treated GPA patients (both in remission) and the same matched HCs as Untreated Cohort 2 (Treated Cohort 2; Table 1). In Table 2 an overview of the aims and experiments performed for each cohort is given.

Table 1. Characteristics of HCs and GPA patients for each cohort.

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AZA: azathioprine; GPA: granulomatosis with polyangiitis; HCs: healthy controls; IS: immunosuppressive; MMF: mycophenolate mofetil; Pred: prednisone

AZA: azathioprine; cANCA: cytoplasmic anti-neutrophil cytoplasmic autoantibody; CRP: c-reactive protein; GPA: granulomatosis with polyangiitis; HCs: healthy controls; IS: immunosuppressive; MMF: mycophenolate mofetil; Pred: prednisone
Flow Cytometry Analysis of B cell Subsets

EDTA venous blood was obtained from patients and HCs and immediately washed twice in PBS with 1% BSA (wash buffer). Next, 100 µl blood was incubated with anti-human CD19-eFluor450, CD27-APC-eFluor780, CD38-PE-Cy7 (eBioscience, San Diego, CA, USA), CD24-FITC, IgM-APC, IgD-PE (BD Biosciences, San Jose, CA, USA) or corresponding isotype controls for 15 minutes and treated with 10x FACS Lysing solution (BD Biosciences) for 10 minutes. After washing, samples were acquired on a FACS LSR-II flow cytometer (BD Biosciences). At least 200,000 events were measured, and plotted using Kaluza v1.5a flow analysis software (Beckman Coulter, Brea, CA, USA). Figure 1A shows a representative gating example.

B cell Proliferation

Peripheral blood mononuclear cells (PBMCs) were isolated, frozen and thawed as described before. PBMCs were washed twice in PBS, followed by a 15-minute incubation at 37°C with eFl670 (eBioscience). Next, PBMCs were cultured in the presence or absence of 500 ng/mL CpG (Hycult Biotech, Uden, the Netherlands), with 3 µM MPA or 3 µM 6-MP (Sigma-Aldrich, St. Louis, MO, USA). After 3 days of culture, samples were washed and stained with anti-human CD19-eFluor 450, CD22-APC (BioLegend, San Diego, CA, USA) and CD3-BV786 (BD Biosciences). At least 200,000 cells were acquired, and plotted using Kaluza v1.5a. In Figure 2A a representative gating example is given.

Determination of in vitro B cell Cytokine Production

To determine the in vitro effects of 6-MP and MPA on B cell cytokine production PBMCs were cultured at a concentration of 1*10^6 cells/mL in the presence of 500 ng/mL CpG alone or in combination with 3 µM MPA or 3 µM 6-MP. After 3 days of culture, PBMCs were restimulated for 5 hours with 2 mM calcium ionophore (Ca-I) and 50 ng/mL phorbol myristate acetate (PMA) in the presence of 10 µg/mL brefeldin A (BFA; Sigma-Aldrich). Next, cells were stained with Zombie Dye NIR (BioLegend) to exclude dead cells. Cells were washed in PBS with 1% BSA (wash buffer) and stained for 15 minutes with anti-human CD19-eFluor 450, CD22-PE-Cy5 (BioLegend), and CD3-BV786. Afterwards, cells were treated with the Fix&Perm kit (Invitrogen, Life Technologies, Grand Island, NY, USA) and incubated with anti-human IL-10-PE, and IL-6-APC and TNFα-AF488 (BioLegend) to stain intracellular cytokines. Samples were acquired on a LSR-II (BD Biosciences) and analyzed in Kaluza v1.5a (Beckman Coulter, Brea, USA). Gates were set per donor on unstimulated sample. A representative gating example is given in Figure 2A.

To assess the influence of AZA and MMF on in vitro B cell cytokine production, PBMCs were cultured at a concentration of 1*10^6 cells/mL in the presence of 500 ng/mL CpG or left unstimulated. The same procedures and materials were used for restimulation.
and staining as described above, except for two monoclonal antibodies: CD22-APC and IL-6-PE-Cy7 (eBioscience). Gates were set per donor on unstimulated sample. A representative gating example is given in Figure 3A.

**Statistical Analysis**
The statistical analysis was performed using Graphpad Prism v7 (GraphPad Software, San Diego, CA, USA). Data are presented as scatter dot plots with median values indicated. Data within the text are represented as median values and range. The Kruskal-Wallis test was used to test for differences between three or more groups for unpaired samples and a Friedman test for paired samples. The Dunn’s multiple comparisons test was used for correcting for multiple testing. P-values of <0.05 were considered statistically significant.

**Results**

**Untreated and MMF-treated GPA Patients Show a Decreased Frequency of Circulating Memory B cells**
First, we determined the frequencies of circulating B cell subsets in HCs and GPA patients that were actively treated with MMF or AZA, and patients not receiving maintenance therapy (untreated and treated patient cohorts 1; Figure 1A). No alterations in B cell subset frequencies were found between the different patient groups (Figure 1B). In comparison to HCs, several differences in B cell subset frequencies were observed in GPA patients irrespective of treatment. AZA-treated GPA patients showed higher frequencies of total B cells and lower frequencies of CD24\(^{hi}\)CD38\(^{hi}\) Bregs, CD24\(^{hi}\)CD27\(^{hi}\) Bregs and transitional B cells compared to HCs. Both MMF-treated patients and untreated patients showed a comparable B cell subset profile: memory B cell and CD24\(^{hi}\)CD27\(^{hi}\) Breg frequencies were decreased, whereas naïve B cell frequencies were increased compared to HCs (Figure 1B).

We also compared the absolute numbers of circulating lymphocytes and B cells between the patient groups. No lymphocyte counts were available for the HC group. Numbers of both lymphocytes and B cells were decreased in treated patients compared to untreated patients, however, no differences were found between MMF- and AZA-treated patients (data not shown).
Figure 1. Memory B cells are decreased in MMF-treated patients and untreated patients compared to HCs. 

A. Representative gating example of the B cell subsets. All gates were set on respective isotype controls. Within CD19⁺ B cells we first gated on CD38⁺CD27⁻ transitional B cells, CD38⁻/dimCD27⁻ naïve B cells, CD38⁻/dimCD27⁺ memory B cells and CD38⁺CD27⁺ plasmablasts using the CD38/CD27 plot. In the CD24/CD38 plot we determined the CD24⁺CD38⁺ Bregs and in the CD24/CD27 plots we gated on CD24⁺CD27⁺ Bregs.

B. The frequencies of all subsets in HCs (open triangles), MMF- (open diamonds) and AZA-treated (open circles) and untreated (filled circles) GPA patients. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, #p<0.1
MPA and 6-MP Inhibit B cell Proliferation *in vitro*

To assess the *in vitro* effect of 6-MP and MPA (active compounds of AZA and MMF, respectively) on B cell proliferation, we cultured CpG-stimulated PBMCs of untreated GPA patients (Untreated cohort 2) and HCs for three days in the presence or absence of these compounds (Figure 2A). Both MPA and 6-MP inhibited B cell proliferation in CpG-stimulated PBMCs from HCs and GPA patients compared to PBMCs stimulated with CpG only (Figure 2B). In HCs, MPA inhibited B cell proliferation to a greater extent as 6-MP, whereas it tended to do this in GPA patients.

Subsequently we determined whether active AZA and MMF treatment had an effect on the capacity of B cells to proliferate (Treated cohort 1). For this purpose, we stimulated PBMCs of AZA- and MMF-treated patients for three days with CpG. As shown in Figure 2C, CpG stimulation of PBMCs from AZA- and MMF-treated patients induced *in vitro* B cell proliferation with frequencies of proliferating B cells being similar to those observed in CpG-stimulated PBMCs from HCs.

![Figure 2. Decreased B cell proliferation upon MPA and 6-MP stimulation.](image)

**Figure 2.** Decreased B cell proliferation upon MPA and 6-MP stimulation. **A.** A representative gating example of B cell proliferation. Within CD19^+^{CD22^+} B cells, B cell proliferation was determined. Gates were set on the unstimulated sample. **B.** B cell proliferation is given for both HCs and GPA patients for the CpG- (open triangle), MPA + CpG (open diamonds), and 6-MP + CpG (open circles) stimulated samples. **C.** B cell proliferation is depicted for HCs (open triangle), MMF-treated (open diamonds) and AZA-treated patients (open circles). #p<0.1, *p<0.5, **p<0.01, ***p<0.001.
Figure 3. MPA decreases B cell IL-6 production. A. Representative gating example of B cell cytokine production. All gates were set on the unstimulated sample per donor. Within CD19^+CD22^+ B cells IL-10^+, IL-6^+ and TNFα^+ B cells were determined. B. The frequencies of all cytokine-positive B cells are depicted for HCs (grey) and GPA patients (black) in CpG only (open triangles), CpG + MPA (open diamonds), and CpG + 6-MP (open circles) stimulated PBMCs. C. IL-10^+, IL-6^+ and TNFα^+ B cell frequencies in HCs (open triangles), MMF-treated (open diamonds) and AZA-treated (open circles) GPA patients. *p<0.05, **p<0.01, ***p<0.001
MPA Decreases the Frequency of IL-6+ B cells \textit{in vitro}

Third, we determined the effect of 6-MP and MPA on B cell cytokine production \textit{in vitro}. PBMCs of GPA patients (Untreated cohort 1) and HCs were stimulated with CpG in the presence of either MPA or 6-MP, and the frequencies of cytokine-producing B cells (IL-10, IL-6, TNFα) were determined (Figure 3A). In PBMC cultures of GPA patients and HCs, MPA significantly decreased IL-6+ B cell frequencies but did not affect the frequencies of TNFα+ B cells compared to samples treated with 6-MP (Figure 3B). Interestingly, MPA reduced the proportion of IL-10+ B cells in PBMC cultures of HCs but not in GPA patients compared to CpG stimulation only (Figure 3B). Addition of 6-MP to the PBMC cultures did not affect the IL-10+, IL-6+ and TNFα+ B cell frequencies in GPA patients.

We next assessed the capacity of B cells from AZA- and MMF-treated GPA patients to produce cytokines. PBMCs of these treated GPA patients (Treated cohort 1) and HCs were stimulated with CpG for 3 days and the frequencies of cytokine-producing B cells (IL-10, IL-6, TNFα) were assessed. As shown in Figure 3C, CpG-stimulated PBMCs from MMF- or AZA-treated patients showed similar IL-10+, IL-6+ or TNFα+ B cell frequencies compared to CpG-stimulated PBMCs from HCs (Figure 3C).

Discussion

AZA and MMF are commonly used drugs to suppress the immune system of patients with autoimmune diseases. These treatments have been shown effective in maintaining disease remission in GPA\textsuperscript{59}. However, these clinical data also show that GPA patients treated with MMF are more prone to disease relapses than AZA-treated GPA patients\textsuperscript{59}. Here, we hypothesized that the difference in relapse rates between MMF- and AZA-treated GPA patients may be due to drug-related differential effects on B cell subset frequencies and/or functioning. To this end, we assessed whether immunomodulation by MMF and AZA altered B cell subset distribution, and whether the active compounds of these drugs differentially affected B cell functions in GPA patients and HCs.

Although we did find several differences in circulating B cell subset frequencies between GPA patients and HCs, no differences were found between AZA- and MMF-treated patients. We subsequently studied B cell proliferation, and showed that both MPA and 6-MP inhibited \textit{in vitro} B cell proliferation in HCs and GPA patients. Interestingly, MPA decreased B cell proliferation in HCs to a greater extent than 6-MP. Moreover, MPA reduced the IL-6+ B cell frequency \textit{in vitro} whereas 6-MP did not. Next, we aimed to confirm these \textit{in vitro} effects on B cell cytokine production by assessing B cell cytokine production and proliferation in PBMC samples from GPA patients receiving AZA or MMF treatment. However, no differential effect on B cell proliferation or cytokine profile was detected between AZA- and MMF-treated patients.
AZA and MMF are known to affect B cell frequencies in the circulation of patients suffering from autoimmune diseases. Our finding that transitional B cell frequencies are decreased in AZA-treated patients is in line with a study of Eickenberg et al.\textsuperscript{180} in AZA-treated SLE patients. However, in contrast to our observations, they also reported decreased circulating memory B cell and plasmablast frequencies in MMF- compared to AZA-treated SLE patients. Eickenberg et al. argued that MMF, in contrast to AZA, spared naïve B cells of SLE patients whereas it profoundly decreased antigen-experienced B cells and their functioning (i.e. antibody production and proliferation)\textsuperscript{180}. Although we did not assess antibody production, this differential effect of MMF on antigen-experienced B cells might also be responsible for the decreased memory CD24\textsuperscript{hi}CD27\textsuperscript{+} Breg frequency in MMF-treated GPA patients observed in our study. However, it is unknown whether these differential effects occur due to direct effects of the drugs on B cells or indirectly due to inhibition of innate immune cells or T cells.

An important feature of AZA and MMF is the inhibition of immune cell proliferation. These immunosuppressive drugs inhibit enzymes of the IMPDH family, which results in cell cycle arrest\textsuperscript{174,184}. MPA specifically inhibits IMPDH2 involved in the activation of lymphocytes\textsuperscript{184}, while AZA inhibits IMPDH that is expressed in all leucocytes. Unexpectedly, we did not observe decreased B cell proliferation in cultured PBMC samples from AZA- and MMF-treated GPA patients (compared to HC) upon CpG stimulation. Previous studies have shown that the active compound of MMF, MPA, is capable of inhibiting T- and B cell proliferation\textsuperscript{181} in HCs \textit{in vitro}. MPA was also capable of halting T- and B cell proliferation in patients with active autoimmune hepatitis\textsuperscript{185} and SLE\textsuperscript{180}, respectively. In contrast, 6-MP only inhibited T cell proliferation at high concentrations (>10 nM)\textsuperscript{60} or not at all\textsuperscript{185}. Here, we show that MPA and 6-MP are both capable of inhibiting B cell proliferation at a concentration of 3 nM. However, we did not observe differences in the proliferative capacity of B cells from AZA- or MMF- treated patients. It is unclear why these differences were not observed; however, one could speculate that \textit{in vitro} concentrations of the active drug compounds differ from concentrations that are reached in the circulation. In this context, a study in autoimmune hepatitis patients reported circulating MPA concentrations of up to 1-3.5 μg/L\textsuperscript{185}. In our \textit{in vitro} studies we used 1 μg/L (=3 nM) MPA, indicating that the applied \textit{in vitro} concentrations are in the range of circulating concentrations reached when patients receive MMF treatment. To date, no data are available for the 6-MP concentrations in the circulation.

We also investigated the effects of both drugs and their active compounds on B cell function by assessing their capacity to secrete cytokines upon CpG stimulation. IL-6 and TNFα are considered to promote the inflammatory response. We showed that neither MPA nor 6-MP affected the TNFα\textsuperscript{+} B cell frequencies. This is in contrast to others who did show that MPA decreased TNFα production in B cell cultures of HCs\textsuperscript{186}. A possible explanation for this discrepancy might be a different set-up of the \textit{in vitro} cultures.
Effects of AZA and MMF on B cells in GPA

Wadia et al. cultured B cells in the presence of a stimulation cocktail, while we cultured PBMCs and stimulated B cells with CpG. Interestingly, we found that MPA decreased IL-6+ B cell frequencies in both GPA patients and HCs, whereas these frequencies were not different in 6-MP treated samples.

To our knowledge this study is the first to investigate the effects of commonly used immunosuppressive therapies on *in vitro* B cell functioning in GPA patients. Previously, a randomized controlled trial reported an increased relapse rate in MMF-treated AAV patients compared to AAV patients treated with AZA59. Our findings indicate that this difference in efficacy between MMF and AZA in GPA is not due to differential effects of these drugs on B cell subset frequencies and/or functioning. The increased relapse rate in MMF-treated AAV patients also contrasts with studies in other autoimmune diseases. In SLE, MMF is known to be equally effective in inducing remission as cyclophosphamide187, while long-term MMF treatment was more effective to maintain remission than AZA188. Also in Crohn’s disease patients, MMF induced disease remission earlier than AZA189.

The current study has several limitations. First, only small groups of patients and HCs were included, and our findings should be validated in larger patient cohorts. Second, we did not consider differences in induction therapy and their effects on the immune system, although included GPA patients were in stable remission for at least 6 months. Third, the *in vitro* concentrations of the active drug compounds, as discussed above, potentially differ from the concentrations that are reached in the circulation. Lastly, we did not assess the effects of both immunosuppressive drugs on serum immunoglobulin or ANCA levels. These could be interesting indicators to link the effect of AZA and MMF on B-cell functioning with their differential effect on relapse rate.

In conclusion, we showed that MPA - the active compound of MMF - inhibits, in contrast to 6-MP, *in vitro* pro-inflammatory B cell cytokine production in GPA patients and HCs, while both compounds inhibited B cell proliferation. GPA patients treated with MMF have an increased relapse rate, however, MMF seems, at least *in vitro*, more effective in inhibiting the pro-inflammatory B cell response of GPA patients compared to AZA. Thus, our results do not provide an explanation for the increased relapse risk of GPA patients treated with MMF. More research is needed to assess the effects of both immunosuppressive drugs on other (pathogenic) immune cells in GPA, to elucidate the immune mechanisms underlying the difference in relapse rates between AZA- and MMF-treated patients and to identify the most effective target for therapy.

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Disclosure Statement
The authors have declared no conflict of interest.