Summary, General Discussion and Future Perspectives
Anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAV) is a rare systemic autoimmune disease characterized by necrotizing inflammation of small- to medium-sized blood vessels. AAV comprises different clinicopathological phenotypes including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic GPA and renal limited necrotizing crescentic glomerulonephritis. ANCA are considered to play an important role in the pathogenesis of AAV. These autoantibodies (autoAbs) are primarily directed against the neutrophil- and monocyte-derived enzymes proteinase (PR) 3 or myeloperoxidase (MPO).

Although the disease etiology is unknown, the pathogenesis of AAV is generally considered to be multifaceted involving genetic predisposition, environmental exposure (e.g. infections) and a complex interplay between multiple immune cells. Particularly T- and B cells belonging to the cellular adaptive immune response play an important role in AAV disease mechanisms. An altered distribution of CD4+ T helper (Th) cell subsets reflected by an expansion of a subset of circulating memory Th cells, termed effector memory T cells (T EM), has been observed in AAV patients in remission. Also, in these patients a disbalance has been demonstrated in circulating Th cell responses with a relative skewing towards Th17 cells as evidenced by increased circulating frequencies of Th17 cells, increased serum interleukin (IL) 17A levels, and an expanded population of pathogenic PR3-specific Th17 cells. Importantly, regulatory T cells (Tregs), characterized by their immune modulating capabilities, are defective in AAV patients. Besides Th cells, B cells are well known to be involved in the pathogenesis of GPA as ANCA-producing cells. Next to their capacity to produce (auto)Abs, B cells are also considered to contribute to autoimmune responses by Ab-independent functions such as antigen (Ag) presentation and cytokine production. Recently, an additional subset of B cells with immunomodulatory capability, termed regulatory B cells (Bregs), has been identified. However, their role in AAV remains largely unexplored.

To suppress the effector cells of the cellular immune compartment, patients with AAV receive standard remission induction and subsequent maintenance immunosuppressive therapies. Two of the commonly used immunosuppressive agents in AAV used as maintenance treatment include mycophenolate mofetil (MMF) and azathioprine (AZA). AZA acts by inhibiting enzymes of the isonine-5’-monophosphate dehydrogenase (IMDP) family thereby inhibiting leucocyte proliferation whereas MMF inhibits IMDP type 2 resulting in inhibition of lymphocyte proliferation. Although it is widely acknowledged that these drugs block immune cell proliferation, the effects of MMF and AZA on the distribution and function of B cell subsets is poorly understood. Of note, it is currently unknown whether differential immunomodulatory effects of MMF and AZA are related to the fact that MMF-treated AAV patients are more prone to future disease relapses than AZA-treated patients. Although immunosuppressive therapies have significantly improved the prognosis of the disease, treatment is often associated with significant...
toxicity, including an enhanced risk of infection, myelosuppression, malignancy, and cardiovascular disease. Therefore, less toxic and more specific treatment is needed for AAV. Previously, B cell-depleting therapy using rituximab has been shown to be as effective as cyclophosphamide for induction of remission in patients with newly diagnosed AAV, despite not targeting (the autoAb-producing) plasma cells\textsuperscript{40,41}. Given that the initial response to rituximab is seen within 72 hours of treatment, it is not likely to be due to reduced Ab production alone\textsuperscript{218}. It is well known that B cell-derived cytokines affect the differentiation of naive into effector Th cells\textsuperscript{219}. Thus, the beneficial effect of rituximab may result from its indirect impact on Th cell responses. Therefore, it is important to investigate the Ab-independent roles of B cells in AAV to reveal potential anomalies in B cell functioning and their effect on Th cells.

Unravelling the role of cellular immunity (involving both B- and T cells) in the pathogenesis of AAV could aid in the discovery of potential biomarkers to predict relapses and the identification of more specific targets for therapeutic intervention. One type of AAV, i.e. GPA, is particularly a relapsing disease in which ±60\% of the patients experience one or multiple relapses during the course of their disease\textsuperscript{61}. Hence, it is essential to identify GPA patients at risk for relapse (already) during disease remission in order to prevent these relapses and there is still a pressing need for novel and more reliable biomarkers that accurately predict disease relapses in GPA patients. Therefore, the aims of this thesis were to:

1. Study the functional role of Bregs and effector B cells in the pathogenesis of GPA
2. Assess the effect of immunosuppressive therapy on B cell functioning in GPA
3. Examine the B cell repertoire as biomarker for future GPA disease relapses

In Chapter 3, we determined the frequency of circulating Bregs (i.e. phenotypically characterized as CD<sub>19</sub><sup>+</sup>CD<sub>24</sub><sup>hi</sup>CD<sub>38</sub><sup>hi</sup> cells). The frequencies of circulating Bregs were significantly decreased in GPA patients receiving immunosuppressive therapy at the time of measurement, whereas these frequencies were not different in untreated GPA patients compared to healthy controls (HCs). Indeed, immunosuppressive treatment is well known to induce lymphopenia in the circulation\textsuperscript{220}. Hence, it is possible that the discrepancy in the literature with respect to Breg frequencies in remission GPA patients (discussed in Chapter 2) exists because patients were not stratified according to immunosuppressive therapy at the time of sampling\textsuperscript{45}. Also, in other autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) numerical Breg deficiencies have been demonstrated, although in both studies untreated and treated patients were included\textsuperscript{131,135}. Functional studies on Bregs have shown that these cells can inhibit Th cell responses\textsuperscript{45,131}. Together with the observations that in several autoimmune diseases frequencies and/or suppressive function of Bregs are reduced\textsuperscript{131,221-225}, this prompted us to study whether
reductions in Breg frequencies in GPA patients had an impact on the distribution of circulating effector Th cells in Chapter 3. To this end, we assessed the frequency of circulating Bregs in relation to the distribution of both circulating Th\textsubscript{EM17} cells (phenotypically characterized as CC-chemokine receptor (CCR) 6\textsuperscript{+} CXC-chemokine receptor (CXCR) 3\textsuperscript{−}CCR4\textsuperscript{+}CCR7\textsuperscript{−}CD45RO\textsuperscript{−}CD4\textsuperscript{+}CD3\textsuperscript{+} cells) and Th\textsubscript{EM1} cells (phenotypically characterized as CCR6\textsuperscript{−}CXCR3\textsuperscript{+}CD4\textsuperscript{−}CCR7\textsuperscript{−}CD45RO\textsuperscript{−}CD4\textsuperscript{+}CD3\textsuperscript{+} cells) in GPA patients. As mentioned above, Breg frequencies were decreased in GPA patients treated with immunosuppressive drugs, whereas these frequencies were not altered in untreated GPA patients compared to HCs. The Th\textsubscript{EM17} cell frequencies were increased in both treated and untreated GPA patients compared to HCs, whereas the Th\textsubscript{EM1} cell frequency was decreased albeit in treated patients only. Importantly, we observed an inverse correlation between Th\textsubscript{EM17} cell and CD24\textsuperscript{hi}CD38\textsuperscript{hi} Breg frequencies in the circulation of untreated GPA patients whereas a positive correlation was found between these Bregs and Th\textsubscript{EM1} cells. No correlations between these cell subsets were found in treated patients. Although associative, these observations suggest that Bregs are important regulators of Th\textsubscript{EM} cells in untreated GPA patients. To investigate whether Bregs were indeed capable of suppressing Th\textsubscript{EM} cell responses, we sorted and co-cultured CD4\textsuperscript{+} Th cells with either CD24\textsuperscript{hi}CD38\textsuperscript{hi} Breg-depleted B cells or with total B cells of GPA patients in remission in the presence of CpG-deoxynucleotides (CpG) and Staphylococcal enterotoxin B. As explained in Figure 1, we showed that depletion of CD24\textsuperscript{hi}CD38\textsuperscript{hi} Bregs resulted in an increased IL-17\textsuperscript{+} Th cell frequency, whereas no changes in IFN\textgamma\textsuperscript{+} Th cell frequencies were detected in GPA patients in remission. These findings are in line with a study in tuberculosis patients, although Bregs in this study were defined as CD19\textsuperscript{−}CD1d\textsuperscript{+}CD5\textsuperscript{+}\textsuperscript{156}.

![Circulation vs. in vitro](image)

**Figure 1.** Disturbed Th\textsubscript{EM17} and Th\textsubscript{EM1} cell frequency distribution within CD4\textsuperscript{+} Th cells and the **in vitro** effect of CD24\textsuperscript{hi}CD38\textsuperscript{hi} Bregs on the Th cell response in GPA patients. **Left:** In the circulation of untreated GPA patients increased Th\textsubscript{EM17} cell frequencies are present, whereas the Th\textsubscript{EM1} cell frequencies are decreased compared to HCs. **Right:** In vitro co-cultures of total B cells or CD24\textsuperscript{hi}CD38\textsuperscript{hi} Breg-depleted B cells with CD4\textsuperscript{+} Th cells of GPA patients showed that Bregs reduced IL-17 production by Th cells but had no effect on IFN\textgamma production.
In support of our findings, studies assessing the B cell repertoire after B cell depletion with rituximab in RA and SLE patients revealed that this treatment reduced the Th17 cell response and restored the Th cell balance. In these patients, B cells with the CD24hiCD38hi phenotype were the first B cell subset that emerged from the bone marrow after B cell depletion with rituximab and became the dominant circulating B cell subset. Indeed, it has been speculated that the effectiveness of rituximab in AAV can in part be ascribed to the expansion of Bregs and subsequent inhibition of Th17 cell responses. Alternatively, a recent study showed that in vitro CD8+ T cell pro-inflammatory cytokine response was decreased in AAV patients treated with rituximab. Moreover, a co-culture of B cells and naive CD8+ T cells of AAV patients resulted in increased pro-inflammatory cytokine production by these T cells compared to HCs. Thus, rituximab treatment might normalize the CD8+ T cell response and during B cell repopulation an enrichment in circulating Bregs may have a therapeutic effect by inhibiting the pathogenic Th17 cells. However, as discussed before, the identification and function of Bregs is, compared to Tregs, less well defined and we are only beginning to understand the functions of these cells. Studies such as ours (Chapter 3) are required to unravel the role of Bregs in autoimmune disease pathogenesis. Possibly, Bregs could ultimately be used for in vitro expansion and autologous transfer to inhibit autoimmune disease development. However, before we can even think of such therapeutic applications, extensive functional studies are required first to really understand Breg biology and behavior in health and disease.

Besides enhancing the suppressive function of Bregs, inhibiting activated effector B cells might be a promising treatment option for GPA patients. Circulating B cells of active GPA patients have been shown to be in an increased state of activation compared to HCs as evidenced by increased CD38 expression. Moreover, it has been recently demonstrated that activated B cells from patients with autoimmune disease (RA and Sjogren's syndrome (SS)) show increased phosphorylation levels of signaling molecules downstream of the B cell receptor (BCR) complex that could potentially serve as therapeutic targets. One of these important molecules in B cells is Bruton’s tyrosine kinase (BTK), which is a pivotal signaling molecule that directly links BCR signaling to B cell effector function. Therapeutic BTK blockade is already widely used to inhibit B cell activation in chronic lymphocytic leukemia patients and has recently been shown to ameliorate kidney disease in a lupus nephritis mouse model. Thus, BTK might be a promising drug candidate for modulating B cell activation and effector function in AAV. In Chapter 4, we examined the BTK protein levels and its phosphorylation status in B cell subsets of GPA patients with active disease and in remission and HCs. We showed that BTK protein levels were significantly increased in transitional and naïve B cells of active GPA patients compared to remission patients and HCs (Figure 2).
addition, increased BCR sensitivity to *in vitro* stimulation with anti-IgM, reflected by increased BTK phosphorylation and signaling, and increased phospholipase C (PLC) γ2 phosphorylation, was detected in active GPA patients. Together, these results indicate that in GPA patients newly emerging B cells are in a heightened state of activation, which is in line with previous work in other autoimmune diseases i.e. RA and SS. We next assessed the impact of a BTK blocker on B cell cytokine production, plasma cell formation and, IgG and ANCA production *in vitro*. The BTK blocker used in our study is a small molecule that binds to the phosphorylation site of the BTK protein, thereby preventing phosphorylation and subsequent activation of the BCR signaling pathway. Upon *in vitro* BTK blockade, we found that the frequencies of IFNγ+, IL-6+ and IL-10+ B cells were decreased in peripheral blood mononuclear cell (PBMC) samples of active and remission GPA patients and HCs, whereas the TNFα+ B cell frequency was only decreased in remission GPA patients. Interestingly, plasma cell formation was only inhibited by the BTK blocker in remission patients, whereas no difference was found in active GPA patients and HCs. Importantly, plasma cell formation was increased in active GPA patients with and without addition of the BTK blocker to the culture compared to remission patients. Lastly, total IgG was decreased in remission patients and HCs in the presence of BTK blockade and seemed to decrease in active patients. Interestingly, PR3-ANCA levels tended to be lower in samples cultured in the presence of the BTK blocker although this did not reach statistical significance, possibly due to low sample size. Breg- and Treg-derived IL-10 is classically known as an anti-inflammatory cytokine capable of suppressing the activation of other immune cells. In *Chapter 4* we found that *in vitro* IL-10+ Breg frequencies were decreased when a BTK inhibitor was added to PBMC cultures. At first glance, such an effect seems undesirable given the well-known anti-inflammatory properties of IL-10. However, IL-10 is also known to exert non-inhibitory functions on various immune cells including B cells (reviewed in ). For example, IL-10 has been shown to inhibit B cell apoptosis, enhance B cell proliferation and differentiation into plasma cells, and promote immunoglobulin class switching. Therefore, in the context of B cell-mediated autoimmune diseases, IL-10 could also be regarded as a pro-inflammatory cytokine. This contention is supported by GWAS studies showing cytosine-adenine repeat polymorphisms in the IL-10 gene of GPA patients, multiple myeloma patients and SLE patients. Functionally, these polymorphisms were associated with increased *in vitro* IL-10 production by lipopolysaccharide stimulated PBMCs of multiple myeloma patients. Moreover, polymorphisms in the IL-10 gene have been reported to correlate with increased autoAb production in SLE patients. Although the functional consequences of IL-10 polymorphisms in GPA remain to be investigated, a recent meta-analysis demonstrated that polymorphisms in the IL-10 gene are associated with susceptibility for development of vasculitis, in particular Behçets disease and GPA. Collectively, these studies clearly indicate the
important role of IL-10 in the pathogenesis of autoimmune diseases. Moreover, we speculate that while IL-10 may be essential for maintaining peripheral tolerance to self-Ags and thus prevent autoimmunity, once tolerance is lost it could in fact promote autoimmune disease by stimulating the differentiation of B cells into autoAb-producing plasma cells.
Based on our in vitro findings the effectiveness of BTK blockade for active GPA patients can be questioned as, for example, plasma cell formation in active GPA patients was not inhibited by the BTK blocker. One possible explanation why BTK blockade did not inhibit plasma cell formation is that B cells of active patients are already in an elevated state of activation and do not require BCR stimulation. Additionally, experimental evidence exists indicating that BTK inhibition not only suppresses B cell activation but also cell migration. The latter was demonstrated in B cell lines where BTK- and PLCγ2-deficient B cell lines were found to be unable to migrate in vitro. Moreover, treatment of primary B cells with BTK or PLCγ2 blockers inhibited B cell migration in vitro indicating that phosphorylation of these proteins is essential in this process. Thus, blocking BTK phosphorylation may be an attractive novel therapeutic strategy for AAV to not only decrease effector B cell functioning but also effector B cell (e.g. memory B cell) migration to sites of inflammation.

The current therapy options for remission maintenance for AAV include rituximab, AZA or MMF combined with glucocorticoids. Interestingly, one open label clinical research trial showed that MMF-treated AAV patients were more prone for disease relapses than AZA-treated AAV patients. Interestingly, the opposite is seen in SLE patients, where MMF is more effective in the maintenance of remission. The effect of MMF and AZA on the immune system, and particularly on B cells, remains poorly understood. We hypothesized that the increased relapse rate in MMF-treated AAV patients may be due to a disbalance in Breg and effector B cell frequencies induced by MMF treatment. Therefore, we investigated in Chapter 5 whether the distribution of Bregs and effector B cells (identified based on their cytokine production) are differentially affected by AZA and MMF in AAV patients. This study consisted of two parts. In part I, PBMCs of untreated remission GPA patients and HCs were stimulated with CpG combined with mycophenolic acid (MpA) or 6-mercaptopurine (6-MP) (i.e. the active compounds of MMF and AZA, respectively) for three days. In part II, B cell functioning was determined in GPA patients that were on maintenance therapy consisting of either AZA or MMF at the time of blood sampling. To do so, we cultured PBMCs of these GPA patients and stimulated the cells with CpG for three days. We showed that B cell proliferation was decreased by MpA and 6-MP compared to CpG only, whereas no difference was found in B cell proliferation in samples of actively treated GPA patients. Interestingly, in vitro stimulation of PBMCs from GPA patients in the presence of MpA showed a decreased IL-6+ B cell frequency compared to 6-MP-treated GPA samples (Figure 3). Importantly, the IL-10+ B cell frequency was decreased by MpA in HC samples only whereas 6-MP did not affect the cytokine-positive B cell frequencies at all. Although these results seem to indicate that the cytokine profile of B cells in MpA-treated samples is shifted towards a less pro-inflammatory state, these results need to be validated in larger patient cohorts.
Additionally, these immunosuppressive drugs are broad inhibitors of immune cell activation not only affecting B cells but other immune cells as well.

Figure 3. B cell cytokine production in PBMCs of remission GPA patients and HCs in vitro upon exposure to 6-MP or MpA and in CpG-stimulated PBMCs of GPA patients receiving AZA or MMF treatment. Top: The effect of CpG stimulation alone on B cell cytokine production. Middle: The effect of in vitro AZA or 6-MP on B cell cytokine production compared to MMF/MpA treatment. Bottom: The effect of in vitro MMF or MpA on B cell cytokine production compared to AZA/6-MP treatment.

After GPA patients have achieved remission, the most important question for the treating clinician is how to prevent a relapse most effectively with the least amount of toxicity. To accomplish this, clinical and/or serological disease related factors need to be identified that reliably predict the risk for disease relapse in each patient. To date, the most extensively investigated disease-related factor in GPA to predict relapse is the
serum PR3-ANCA titer and changes herein over time. However, results of these studies are inconsistent and the value of serial PR3-ANCA measurements in clinical practice has been a matter of debate. A persistently positive ANCA titer during disease remission has been reported to be associated with future disease relapses\textsuperscript{242}, whereas others found that rather a rise in serum PR3-ANCA titer over time preceded a relapse\textsuperscript{64}. In contrast, others did not find an association between serum PR3-ANCA levels and ensuing disease relapses\textsuperscript{67}. Indeed, a meta-analysis by Tomasson \textit{et al.}\textsuperscript{68} showed that changes in serum PR3-ANCA titer over time are only weak predictors of disease relapses and are ineffective in predicting disease relapses for all AAV patients. However, more recent research suggests that serial PR3-ANCA measurements may be helpful in identifying relapses in patients presenting with renal involvement and alveolar hemorrhage\textsuperscript{66,243}. Additionally, PR3-ANCA levels should also be determined using novel methods (e.g. by Phadia analyzer) to investigate whether such methods are more sensitive in detecting rises in PR3-ANCA levels which may improve relapse prediction based on changes in ANCA levels.

It has also been proposed that the prevalence of particular B cell subsets in the circulation of AAV patients in remission may be an alternative disease related indicator for relapse risk. The introduction of rituximab treatment particularly allowed for a unique way to investigate whether specific distribution profiles of repopulated peripheral B cell subsets associate with relapses\textsuperscript{28}. Repopulation of circulating memory B cells at six months post last rituximab infusion was found to be associated with increased relapse risk, whereas patients with naïve B cell repopulation experienced less relapses\textsuperscript{244}. Interestingly, another study showed that increased proportions of circulating CD5\textsuperscript{+} Bregs upon B cell repopulation in AAV patients were related to prolonged remission whereas patients with lower CD5\textsuperscript{+} Breg frequencies showed a shorter time to relapse\textsuperscript{144}. However, not all GPA patients are treated with rituximab. Thus, in Chapter 6 we examined whether B cell subset frequencies could predict future disease relapses in GPA patients in remission not treated with rituximab. We showed that an increased frequency of circulating plasmablasts was associated with decreased relapse-free survival. Circulating plasmablast frequencies showed a trend towards a decrease in the last blood sample collected 1-6 months prior to the relapse, which might indicate plasmablast migration to sites of inflammation in GPA. Therefore, we stained plasmablasts in kidney biopsies and urine of active AAV patients with renal involvement. Together, these results indeed suggest that plasmablasts migrate from the circulation to sites of inflammation and that monitoring circulating plasmablast frequencies might be a useful indicator for future disease activity.

Plasmablasts are a result of the germinal center reaction and are migrating to plasma cell niches mainly in the bone marrow to become plasma cells. In contrast to plasma cells, plasmablasts are capable of producing only low amounts of antibodies. Plasmablasts
are, like plasma cells, typically present in low numbers in the circulation. Importantly, in other autoimmune diseases increased plasmablast frequencies were found to correlate with autoAb levels and disease activity. The data presented in Chapter 6 suggest that in patients with upcoming relapses, B cells are already activated and instructed to differentiate towards plasmablasts. The decrease in plasmablasts in the last sample before relapse might highlight differentiation into plasma cells and migration of these cells to sites of inflammation. Although plasmablasts were related to decreased relapse free survival when present in higher frequencies, we did not detect a correlation between plasmablast frequencies and ANCA titers. Nonetheless, others have shown that both memory B cells and plasmablasts are the predominant B cell subsets of GPA patients to react with PR3. Recently, PR3-ANCA-positive B cells have been detected in inflamed tissues of GPA patients which is in line with our view that autoreactive B cells migrate from the circulation to sites of inflammation. However, it is currently not known whether plasmablasts at sites of inflammation can secrete PR3-ANCA.

Figure 4. Plasmablasts as indicators for future disease relapses. During remission (top), future relapsing GPA patients demonstrated with increased plasmablast frequencies. During active disease (bottom), plasmablast frequencies were increased in the urine of GPA patients with renal involvement, whereas this frequency was lower in peripheral blood. Additionally, plasmablasts infiltrated the inflamed kidney in these patients.

Although plasmablast frequencies might provide a novel marker to identify patients at risk for relapse, a predictive B cell marker at an earlier stage of the disease is highly
preferred. Plasmablasts are already at a “late” differentiation stage and are in the process of becoming Ab-producing plasma cells. Ideally, in AAV, but also in other autoimmune diseases, the formation of pathogenic autoAb-producing cells is prevented. This would be possible if patients at risk for relapse are identified earlier during disease remission but this requires a marker that indicates increased activation of B cells at earlier differentiation stages. BTK protein and its phosphorylation levels in newly emerging B cells might provide such a B cell-specific early marker. In Chapter 4 we showed that BTK levels were increased in transitional and naïve B cells of active patients only. Importantly, these increased BTK levels correlated with B cell activation. Although in our study BTK levels were not increased in remission GPA patients, it is likely that B cells become increasingly activated in patients that are about to relapse. Currently, data on BTK protein and phosphorylation levels in newly emerging transitional and naïve B cells of remission patients with approaching relapse is lacking. This is however of interest because if newly emerging B cells in remission patients with future relapses indeed show increased BTK levels, it would indicate increased B cell activation and might identify patients at risk for relapse. The possibility of measuring B cell BTK levels as an indicator for future relapse should be tested in a larger cohort with multiple fixed time points to analyze changes in BTK levels over time.

**Future Perspectives**

The work presented in this thesis contributes to our knowledge on the role of B cells in the pathogenesis of GPA and explored their potential as target for novel treatment strategies and predictors of relapses. We have studied the distribution of B cell subsets in GPA patients and found that an increased frequency of circulating plasmablasts was associated with decreased relapse-free survival. Moreover, plasmablast frequencies were found to be decreased in blood samples collected 1-6 months prior to relapse of the disease and these cells could be detected in kidney biopsies and urine of GPA patients with active renal disease. To this end, analyses of B cell subset distribution and monitoring the frequency of plasmablasts in blood and urine could be informative as an indicator of disease status and possibly aid in the recognition of an upcoming relapse in AAV patients. Future studies in larger patient cohorts, including a cohort of rituximab-treated GPA patients, are however necessary to substantiate whether plasmablast frequencies are indeed predictive of relapses in GPA and should determine whether these include plasmablasts that produce ANCA. Such studies will open up new avenues for future use of these cells as markers for (upcoming) disease activity or as targets for novel therapeutic strategies.

We also investigated the association between Bregs and the expanded Th17 cell response in GPA patients. We observed an inverse correlation between circulating Bregs
and Th$_{17}$ cells in GPA patients, and in vitro Breg depletion resulted in an increased Th17 cell frequency. Thus, a reduction of circulating Bregs in GPA patients may contribute to increased numbers of Th17 cells which release IL-17, a pro-inflammatory cytokine implicated in cell migration and granuloma formation in GPA patients. Future studies are required to identify the signals that induce Breg expansion, as this may provide clues to develop novel strategies to control Th17 cell responses in GPA patients and perhaps autoimmune diseases in general. Moreover, it is essential that consensus is reached on Breg phenotype(s), e.g. based on (novel) surface or functional markers or combinations thereof, to truly discover the regulatory potential of these cells. Only then the potential of therapeutic Breg transfer upon ex vivo expansion can be investigated as a possible treatment strategy to restore immune balance.

In addition to the disturbed B cell subset distribution, we demonstrated alterations in the BCR signaling pathway in newly emerging transitional and naive B cells of active GPA patients. These B cells showed increased BTK levels whereas blocking BTK activity inhibited B cell cytokine and IgG production, and plasma cell formation in vitro. Hence, BTK might be a novel therapeutic target to dampen B cell activation in GPA patients and future preclinical and clinical trials should establish whether BTK is a potential novel treatment option for these patients. Our findings on intracellular BCR signaling molecules such as BTK may also hold promise for the discovery of novel biomarkers for (upcoming) disease activity. To investigate the potential of BTK as a biomarker, the dynamics of BTK expression in B cells should be investigated in larger (longitudinal) studies of future-relapsing and non-relapsing AAV patients.

Classically, the therapeutic strategy for GPA consists of remission induction and maintenance therapy using immunosuppressive medication. However, B cell depletion therapy by rituximab is increasingly applied for remission induction and maintenance in GPA patients. Although rituximab is an efficacious therapeutic strategy, it is not specific for the autoreactive B cells since it depletes all B cells including Bregs. The suggested Breg expansion or BTK blockade might provide an additional therapeutic strategy for AAV, and this could be more specific by inhibiting Th17 cell activation and B cell activation, respectively.

However, the most ideal therapy for autoAb mediated autoimmune diseases would be one that specifically depletes autoreactive B cells only. Interestingly, Ellebrecht et al. tested such a novel therapy to deplete autoreactive B cells in pemphigus vulgaris (PV). PV is a severe autoimmune disease that involves blistering of the skin and oral mucosa and is characterized by autoAbs directed against keratinocyte adhesion proteins (e.g. Dsg3). The authors engineered T cells with a chimeric autoAg receptor (CAAR) and showed that these CAAR T cells specifically eliminated autoreactive Dsg3-specific B cells in vitro and in a PV mouse model. This elegant study provides proof of principle that this form of cellular immunotherapy might be a future approach to treat...
autoimmune diseases. The fact that this arising therapy specifically targets autoreactive B cells makes it a very promising research field that could ultimately lead to an effective and durable treatment of AAV and other autoimmune diseases.
References


Chapter 7


