7

Thesis summary and discussion
Summary

Giant cell arteritis (GCA) is the most frequent form of an inflammatory vasculitis affecting medium and large-size arteries in people older than 50 years. Two forms of GCA can be distinguished according to the location of the affected blood vessels: Cranial (C)-GCA, with involvement of the cranial vessels (including temporal artery), and Large Vessel (LV)-GCA, with involvement of the aorta and its proximal branches. Symptoms may differ, as C-GCA presents mostly with headache, jaw claudication and/or visual loss while LV-GCA presents mostly with non-specific symptoms such as low-grade fever and weight loss. GCA can lead to blindness, stroke or aortic aneurysm/dissection. The disease frequently overlaps with polymyalgia rheumatica (PMR). PMR is characterized by synovitis/bursitis of the shoulders and hips. Both diseases are mainly treated with glucocorticoids (GC). As relapses are frequent, high cumulative doses of GC are often necessary and come with severe side effects. Thus, there is a high unmet need for other treatment options in GCA and PMR.

The pathogenesis of GCA and PMR is still incompletely understood but in the present pathogenetic model both diseases are regarded as T cell and macrophage mediated diseases. Temporal artery biopsies of patients with GCA indeed show extensive infiltration of T cells and macrophages. On the contrary, B cells are infrequently observed in the temporal artery biopsy. This finding together with the lack of disease-specific autoantibodies in GCA explains the limited interest in B cells in these diseases in the past. Only recently, an altered B cell homeostasis was reported in newly diagnosed GCA and PMR patients. Although this is a first clue that B cells could participate in disease pathology, their exact role is still unclear. B cells can differentiate in antibody-secreting plasma cells but can also contribute to diseases via antibody independent functions such as cytokine production.

In this thesis, we aimed to explore the role of B cells in the pathogenesis of GCA and PMR with a focus on their cytokine production. We studied the distribution of circulating B cell subsets in both GCA and PMR. We also investigated the location and organization of B cells within the inflamed arterial wall and studied chemoattracting factors involved in the migration of B cells. Lastly, we studied the anti- and pro-inflammatory cytokine production by circulating and lesional B cells.

Chapter 2 comprises a study of leukocyte subsets in a prospectively followed long-term cohort of newly diagnosed GCA and PMR patients as compared to HC. Leukocyte subset counts from peripheral blood obtained before, during and after GC-treatment were compared with HC and infection control (INF) patients. Treatment-naive GCA and PMR patients demonstrated an altered leukocyte composition with lowered B-cell and NK-cell counts but elevated neutrophil and monocyte counts, suggesting a shift towards the myeloid lineage. This myeloid shift was not amended by GC-treatment and persisted
well into treatment-free remission. Our finding may reflect an ongoing subclinical inflammation or alternatively be a sign of cellular senescence of the immune system predisposing patients for disease. Peripheral B cells showed the most fluctuating course over time: lowered in treatment-naïve patients with active disease, but increased after 3 months of GC-treatment followed by a gradual decrease over time upon chronic GC-treatment. In addition, a negative correlation was observed between B cell counts and ESR in treatment-free remission patients. The interpretation of this finding is yet unclear, as it could indicate both a protective effect of the circulating B cells or a pathological role of the B cells that migrated to the site of inflammation to support (sub)clinical active disease.

In Chapter 3 and 4, we performed histopathological studies on tissue-migrated B cells in the inflamed vessel wall of both C-GCA (temporal artery) and LV-GCA (aorta). B-cell presence varied in temporal artery tissue where some arteries did not show any B cell infiltration while others showed multiple B cells organised into clusters. B cells were mostly observed in the adventitial layer of the vessel wall in the proximity of the vasa vasorum. In 33% of the studied temporal artery biopsies, B cells were organized into artery tertiary lymphoid organs (ATLOs). In contrast to the temporal artery where T cells outnumber B-cells, the aorta of LV-GCA patients contained massive amounts of infiltrating B cells in the adventitia. In the majority of LV-GCA patients (78%) these infiltrates were organized into well-defined ATLOs containing germinal centers with proliferating B cells, plasma cell niches and high endothelial venules. In comparison to aortic tissue from atherosclerotic patients, we showed that aorta tissue from LV-GCA contained a higher number of ATLOs and more often also contained a germinal center. Interestingly, approximately half of the ATLOs were located adjacent to a granuloma in the media layer of the vessel indicating a possible communication between the cells of the ATLO and the granuloma, known to consist of T cells and macrophages. In some of the aortic tissues, we also observed B cells at the site of the granuloma in the media layer of the vessel wall.

B cells have proven to be a dynamic cell population during the disease course in both GCA and PMR patients, suggesting B cell trafficking between peripheral and vascular tissues. To learn more about the mechanisms governing B cell trafficking in GCA and PMR, we studied in chapter 5 chemokines and chemokine receptor expression by B-cells in peripheral blood and at the level of the inflamed artery. We observed that the chemokines CXCL9 and CXCL13 were significantly increased in the circulation of treatment-naïve GCA and PMR patients. CXCL13 correlated with disease activity markers and was the only chemokine that increased even further in circulation after 3 months of GC-treatment. Circulating frequencies of CXCR3+ and CXCR5+ memory B cells were lowered and correlated inversely with their complementary chemokines CXCL9 and CXCL13. At the arterial lesions in GCA, we detected CXCR3+ and CXCR5+ B cells localized...
in areas with the expression of CXCL9 and CXCL13. These results suggest an ongoing 
tissue infiltrating migratory process. Of note, we did not observe differences between 
LV-GCA and C-GCA in the studied chemokines and chemokine receptors expression 
that could explain the difference in B cell presence between the aorta and the temporal 
artery. As we did not study synovial tissue from PMR patients we do not know if B 
cells also migrate towards inflamed synovial tissue in PMR. Taken together, especially 
the CXCL9-CXCR3 and CXCL13-CXCR5 axes seem to be important mediators of B cell 
migration in GCA and PMR.

As CXCR3+ and CXCR5+ B-cells infiltrate the inflamed artery in GCA and organize into 
ATLOs, we sought to learn more about the functions of these B cells. This was inspired 
by the notion that the antibody independent roles of B cells, e.g., cytokine production, 
have proven to be important in other autoimmune diseases. Moreover, B cell depletion 
therapy has shown beneficial effects in several autoimmune diseases. In Chapter 6, 
we therefore aimed to assess if cytokine producing B cells are active players in the 
vasculopathy of GCA by studying pro- and anti-inflammatory cytokine production of 
arterial and circulating B cells and comparing this to HC and PMR patients. We found 
that B cells in the GCA artery tissue expressed IL-6, GM-CSF, TNFa, IFNg, LTb and IL-10. 
In addition, circulating B cells of treatment naïve GCA patients showed an increased 
potency to produce pro-inflammatory cytokines. Following in vitro stimulation, 
increased frequencies of IL-6+ and TNFa+IL-6+ B cells were detected in GCA compared to 
HC. These TNFa+IL-6+ B cells were strongly correlated with disease activity marker ESR. 
This correlation was specific for GCA patients as it was not observed in PMR patients. 
Glucocorticoids (GC)-treatment diminished the frequencies of IL-6, GM-CSF, IFNg, IL-2 
and TNFa+IL-6+ cytokine producing B cells. To assess a further role for B cell cytokine 
secretion in the pathology of GCA, we investigated whether B cell cytokine secretion can 
activate and polarize myeloid cells. With an in vitro set-up, we determined that B cell-
conditioned medium leads to macrophage polarization. These polarized macrophages 
produced pro-inflammatory cytokines IL-6, IL-1β, TNFa, IL-23, which are known to play 
a role in the vasculopathy of GCA. Moreover, these macrophages also produced factors 
involved in the breakdown of the vessel wall (MMP9) and in neoangiogenesis (YKL40) 
fitting with the phenotype of CD206+MMP9+ macrophages which are located in the 
media layer and are associated with tissue destruction and neovascularization.
Discussion and future perspectives

Multiple immune and tissue resident cell types are involved in the complex process that forms the pathogenesis of giant cell arteritis (GCA). While T cells and macrophages have received much attention, B cells have only recently emerged as possible contributors in the pathogenesis of GCA. B cells are well known for their capacity to differentiate into antibody secreting plasma cells but less known for their cytokine production. Through cytokine production, B cells can exert both regulatory and effector functions. This thesis is devoted to investigate B cell participation in GCA pathogenesis with a focus on antibody independent, cytokine-mediated B cell functions. We also included patients with polymyalgia rheumatica (PMR), as GCA frequently overlaps with PMR, thus implying overlapping pathogenic pathways. The studies in this thesis are based on peripheral blood and tissue biopsies of the temporal artery and aorta of GCA patients. For PMR the data is limited to peripheral blood as we did not have access to biopsies of the synovial tissue from PMR patients. Peripheral blood can be informative on the disease activity status of patients and is easily obtained. In addition, it is possible to collect blood at multiple timepoints from the same patients thereby enabling longitudinal follow-up studies. However, tissue biopsies of the actual site of inflammation (i.e. temporal artery and aorta in GCA) provide concrete information about the disease pathology. Unfortunately, biopsies only inform on the inflammation at one specific time point in the disease course. Here we discuss the disease-associated changes of B cells in GCA and PMR and the variability in B cells amongst the GCA and PMR patients. Moreover, we propose a new disease model that includes B cells in GCA and discuss the possibility of treating GCA and PMR patients with B cell targeted therapy.

What are the causes of variability in B cell presence and organization?

Early GCA studies investigating the temporal artery reported that few if any B cells were present amongst the infiltrating immune cells. In addition, the limited data on PMR biopsies describe no B cells and varying presence of plasma cells. In contrast with these early studies, more recent data showed that B cells can be present in the temporal artery albeit in lower numbers than T cells and macrophages. This discrepancy on B cell presence in the temporal artery of GCA patients already hints at variability in B cells at the site of vascular inflammation in GCA.

We studied B cell presence in 21 temporal arteries and observed B cells in all but one patient (chapter 3). The number of B cells was indeed highly variable and ranged from a few scattered B cells to B cell clusters in 33% of the studied biopsies. Pathologists usually examine several interval sections of the same temporal artery biopsy to prevent false negative diagnosis due to skip lesions. There was some variation in B cell presence
between sections of the same temporal artery as well. This adds to the notion that there is variability in B cell presence in the temporal artery even within the same patient. In addition, while we observed all B cell clusters in the adventitial layer, others reported on ATLOs in the media layer of the temporal artery\(^{10}\).

We are the first group to study the presence of B cells in the aorta of LV-GCA patients (chapter 4). The aortic tissue segments were obtained from LV-GCA patients who underwent surgery due to late-stage symptoms e.g., aortic aneurysm or aortic dissection. B cells were abundantly present within the aorta, outnumbered T cells, and were mostly organized into well-defined ATLOs. Specifically, 8 out of the 9 studied LV-GCA aorta’s contained B cells and 7 of these aortic tissues contained multiple organized B cells or ATLOs. Thus, in addition to the variability in B cell presence between patients in the temporal artery, there is also a large difference in B cell presence and organization between vascular beds of the temporal artery and the aorta in GCA.

The exact reason for the varying results on B cell presence is unknown but could be related to the differences between vascular beds. The temporal artery is a medium-sized vessel whereas the aorta is a large-sized vessel. Although both vessels are anatomically similar, comprising the intima, media and adventitia layers, the response to GCA induced inflammation appears to be different. Both arteries contain granuloma’s consisting of macrophages and T cells that cause damage in the media layer\(^{12}\). Late-stage complication of inflammation in the temporal artery is vessel occlusion due to intima hyperplasia\(^{13}\). Rather than intima hyperplasia, long-term complications of GCA in aorta are aortic aneurysms. We indeed observed ‘necrosis blocks’ in the media layer of GCA aorta. These are areas that have infiltrating macrophages and T cells on both sides in the media and wherein the tissue structure of vascular smooth muscle cells is completely destroyed. At these locations, the vessel wall is weakened, explaining the tendency for dilation and eventual rupture of the aorta in GCA. Our findings add to the limited knowledge on the pathology of LV-GCA. Specifically for the B cells, the differences between the findings in the temporal artery and the aorta may also be related to differences in the chemotactic mechanisms in C-GCA and LV-GCA or to disease duration.

We investigated whether the variability in B cell numbers and organization between C-GCA and LV-GCA could be explained by differences in peripheral chemokine and chemokine receptor expression on B cells (chapter 5). However, we did not find any differences in serum chemokines and in chemokine receptor expression by B cells or in numbers of circulating B cells between patients with C-GCA and LV-GCA. Thus, although we cannot completely exclude possible differences in local chemokine gradients, these were not reflected in serum chemokine levels. In addition, chemokines and chemokine receptors were also similarly expressed in the GCA affected temporal artery and aorta.

Alternatively, the variability in B cells in the temporal artery and aorta could be explained by the duration of disease. The diagnosis of GCA is based on clinical symptoms
supported by elevated acute phase response markers ESR and CRP\textsuperscript{14,15}. C-GCA patients present with specific symptoms such as a new onset headache, visual disturbances or jaw claudication\textsuperscript{16}. These specific symptoms make patients to consult a physician early after the onset of these complaints. Temporal artery biopsies taken at this time often reveal an already well-developed vasculitis. In contrast to C-GCA patients, the LV-GCA patients experience non-specific symptoms, such as malaise, weight loss and fever, or signs related to vascular damage such as limb claudication, that make diagnosis more difficult and leads to diagnosis delay\textsuperscript{17}. Diagnosis of LV-GCA is sometimes even missed and only established by histopathological analysis in patients who undergo surgery for an aortic aneurysm or dissection. Thus, the LV-GCA aortic aneurysm tissue likely represents a late-stage image of GCA, while the temporal artery depicts an earlier stage. Of note, the studied tissues do not represent the early-stage disease, challenging studies into disease initiation and early pathogenic events. Furthermore, disease duration is also difficult to assess in LV-GCA. The ATLOs, which are most frequently observed in the aorta, also indicate that the aortic GCA tissue depicts chronic inflammation as it is known that ATLOs arise in long-standing inflammation\textsuperscript{18}. This data also suggests that C-GCA patients with well-developed ATLOs in the temporal artery possibly have a longer disease duration compared to temporal artery without the presence of ATLOs. However, others reported that there is no correlation between the number of ATLOs and duration of GCA symptoms prior to temporal artery biopsy\textsuperscript{10}. We also did not detect a correlation between the presence of ATLOs and disease duration in C-GCA patients (chapter 3). More research is needed to investigate whether ATLO formation is related to disease duration.

Data on circulating B cells also show variations. It was already reported that in both GCA and PMR, B cells tend to be lowered in newly-diagnosed, treatment-naïve patients\textsuperscript{9,19}. We indeed found a significant lowering of B cells in newly-diagnosed PMR patients and only a trend for lower B cells in GCA patients (chapter 2). Also, in chapter 5 the circulating B cells of both GCA and PMR patients did show a lowering but this failed to reach statistical significance. These results imply that there is individual variation in circulating B cells numbers between patients. It was previously proposed that B cells migrate towards the inflamed tissue during active disease and return to circulation upon treatment in GCA/PMR patients. This hypothesis is derived from data showing a lowering in circulating B cells in treatment-naïve GCA/PMR patients followed by an increase after 3 months of treatment\textsuperscript{9}. This increase was reportedly not due to compensatory hyperproliferation or enhanced bone marrow production, strongly indicating that the increase is caused by B cells returning from the inflamed tissues or by demargination\textsuperscript{9}. We confirmed these fluctuations in circulating B cells in the extended GPS cohort (chapter 2 and 5). It could be that the variations observed in circulating B cells also reflect the varying presence of B cells in the inflamed arteries. A study analyzing paired biopsies and peripheral blood of patients with GCA could shed light on the mechanisms involved in these differences.
Based on cell surface marker expression, transitional (CD38⁺ CD27⁻), naïve (CD38⁻/⁻ CD27⁻), unswitched memory (CD27⁺ IgD⁺ IgM⁺), switched memory (CD27⁺ IgD⁻ IgM⁻), IgM only memory (CD27⁺ IgD⁻ IgM⁺), double negative (IgD⁻ CD27⁻) memory B cells and plasmablasts (CD38⁺ CD27⁺) are defined. B cell subset distribution was previously examined in GCA and PMR patients combined. We here assessed the B cell subset distribution in GCA and PMR patients separately and observed that these are different. In newly diagnosed GCA patients we found lowered switched memory B cells whereas PMR patients show elevated double negative B cells and plasmablasts. GC-treatment almost depleted transitional B cells in both patient groups while only in GCA patients all memory B cell subsets increased. These memory B cells are likely demarginated B cells or B cells from the inflamed artery tissues. The differences between GCA and PMR in circulating B cells are intriguing and could suggest that B cells may not play the same role in these overlapping diseases. Double negative B cells are for instance documented to be increased in several auto-immune diseases and are related to aging and senescence. It would be interesting to investigate the role of double negative B cells in PMR patients as opposed to GCA patients.

Comparing the possible functions of B cells in GCA and PMR is complicated by the limited knowledge about the presence of B cells in the inflamed synovial tissue or synovial fluid of PMR patients. Our research group is currently conducting a biopsy study in PMR patients (the PROMIS study). It will be interesting to see if B cells are present and if they also organize into ATLOs. As circulating B cells in PMR patients are lowered during active disease, and return upon treatment (chapter 2), it is highly interesting to assess their location during active disease. Next to the site of inflammation, B cells could also migrate towards lymphoid tissue or reside in the marginal pool. Specific B cell tracers (such as CD20) in combination with whole body imaging could reveal the location of the B cells in GCA and PMR patients with active and quiescent disease. This technique will also allow a follow-up study of the B cell dynamics in these patients.

Is there a role for B cells in the immunopathology of GCA and PMR?

Unravelling the pathogenesis of GCA and PMR is an ongoing process. In the current pathogenesis model, B cells are not included. This is due to the low number of B cells found in older histopathology studies and to the lack of disease-specific autoantibodies in GCA and PMR. In this thesis, we have focused on a possible antibody-independent role of B cells and found evidence supporting a cytokine-mediated role for B cells in these diseases. The variability of B cells presence as described above, i.e., B cells are not always present at the site of inflammation or altered in circulation, suggests that B cells are likely not necessary for disease development. Rather, infiltrating B cells are likely to play a role in perpetuation of inflammation. Therefore, we propose to update the current disease model with B cells as contributors to vascular pathology (see figure 1). As the knowledge
about B cells in the inflamed synovial tissue of PMR patients is very limited, this part focuses only on GCA pathogenesis.

**Figure 1.** A schematic representation of a GCA affected temporal artery and aorta. The figure shows the current model without B cells (left side) and the proposed new model including B cells (right side). In the temporal artery B cells are present in varying degrees in the inflamed parts of the vessel wall. B cells are mainly located in the adventitial layer and can be scattered or organized. In the aorta, B cells are also found in the adventitial layer and often organize into well-defined ATLOs with germinal centers, plasma cell niches and high endothelial venules (HEVs). B cells co-localize with several chemokines (CXCL9, CXCL13) and also express the corresponding receptors (CXCR3, CXCR5). B cells likely participate in attracting new cells to the inflamed vessel through either the vasa vasorum or HEV by creating chemotactic gradients. B cells also produce several, mostly pro-inflammatory, cytokines (IL-6, GM-CSF, TNFα, IFNγ) that can polarize macrophages to acquire a tissue destructive and pro-angiogenic phenotype. The anti-inflammatory cytokine IL-10 is also expressed by some B cells. LTβ, a cytokine known for its role in the organization of ATLOs, is indeed strongly expressed at the ATLO sites. With these functions, B cells likely participate in the perpetuation of inflammation in GCA.

It is difficult to establish when exactly in the disease process B cells enter the vessel wall and become a contributing factor. T cells and macrophages are the leukocytes that breach the immune privilege of the vessel wall. These cells migrate from the circulation to the vessel wall in response to chemokines. The chemokine CXCL9 is likely a major contributor to the migration of infiltrating leukocytes and binds to the CXCR3 receptor. It is already known that the CXCR3 receptor and its ligands (CXCL9, CXCL10 and CXCL11) are increased...
in temporal artery biopsies of GCA patients\textsuperscript{26}. B cells can also make use of the CXCL9-CXCR3 axis for migration\textsuperscript{27}. We indeed show that circulating and arterial B cells from GCA patients express the CXCR3 receptor and are located in areas with CXCL9 expression in the vessel wall (chapter 5). This strongly implies that B cells use the CXCL9-CXCR3 pathway for migration to the vessel and could therefore enter the vessel wall at the same time as T cells and macrophages. We also found CXCL13 in the B cell rich areas and documented CXCR5 expression on local and circulating B cells. Ciccia et al. have also reported on increased mRNA levels for CXCL13 and CXCR5 in GCA temporal arteries that contained ATLOs\textsuperscript{10}. The CXCL13-CXCR5 axis is known to be involved in migration towards lymphoid tissue and can play a role in the formation of ATLOs and in directing B cells to the B cell follicles\textsuperscript{28,29}. Interestingly, we also observed that CXCL13 was increased in treatment-naïve GCA and PMR patients and, in contrast to other chemokines, increased even further after 3 months of GC-treatment. In addition, we found a correlation between CXCL13 and disease activity markers in both CGA and PMR at baseline (chapter 5). CXCL13 has been associated with an unfavourable disease course in multiple diseases and is considered a marker for germinal center activity\textsuperscript{28,30–34}. The ATLOs we observed in the inflamed arteries with a follicular dendritic cell network are a likely source of CXCL13.

To learn more about the dynamics of CXCL13 and assess possible usage as a disease monitoring or biomarker, a long-term follow-up analysis, including treatment-free remission patients should be performed.

Infiltrating T cells and macrophages form granulomas that cause vessel wall breakdown, chronic damage and cell death\textsuperscript{25,35}. Possibly, B cells are attracted to the vessel wall during this process in order to enable a quick and local immune response to clean up the damage, coordinated by ATLOs with active germinal centers (chapter 2 and 3). The germinal center reactions could result in antibodies directed against the products of the damaged vessel wall. This fits with the previous findings of auto-antibodies targeted against human proteins, including parts of the cytoskeleton, the proteome of vascular smooth muscle cells or endothelial cells\textsuperscript{36–38}. The presence of these auto-antibodies may thus originate as a consequence of the damage rather than being part of an initiating disease specific pathogenic mechanism.

B cells likely enter the inflamed vessel in the same fashion as other leukocytes, through the vasa vasorum in the adventitia and guided by chemokine gradients. Here, they encounter an inflamed vessel and are influenced by the local chemokine and cytokine milieu created by tissue resident cells and the infiltrating inflammatory cells, especially macrophages, Th1 cells and Th17 cells\textsuperscript{25,35}. The B cells start organizing into ATLOs and express various chemokines and cytokines. The expression of the chemokines CXCL9 and CXCL13 (chapter 5), together with the presence of high endothelial venules at the site of ATLOs (chapter 3), suggest that ATLOs play a role in attracting new naïve cells from the blood stream into the vessel wall, comparable to their behaviour in the lymph nodes\textsuperscript{29}. ATLOs are also reported in other diseases with chronic inflammation, such as
atherosclerosis, and could be referred to as the oval office of immunity because of their regulation of local immune responses\textsuperscript{39–41}.

Next to attracting cells to the site of inflammation, B cells also play a role in polarizing the naïve cells. Leukocytes are thought to enter the vessel wall in an outside to inside manner. This means that macrophages and T cells enter the adventitia through the vasa vasorum (or HEV) (outside) and migrate towards the intima media border (inside) which is the site of granulomas and the site of damage of the internal elastic lamina. The B cells are mainly located in the adventitia and express pro-inflammatory cytokines such as IL-6, GM-CSF, TNFα and IFNγ (chapter 6). Although some B cells also express the anti-inflammatory cytokine IL-10, the overall cytokine production by B cells seems to be biased towards pro-inflammatory cytokines. Thus, infiltrating leukocytes in the adventitia could immediately be polarized by the B cell influenced local cytokine milieu. We indeed show that B cell conditioned medium can polarize macrophages to acquire a tissue destructive and pro-angiogenic phenotype that has been reported to be present in the inflamed GCA arteries\textsuperscript{12}. Others have also proven that especially the combination of IL-6, GM-CSF, IFNγ, and TNFα is highly effective in activating macrophages\textsuperscript{42–44}. The cytokines produced by B cells in GCA likely also skew infiltrating T cells. Although we did not focus on T cells, it is already known that B cells can regulate T cell differentiation by for instance promoting Th17 through IL-6\textsuperscript{45}. Arterial B cells thus produce chemokines and cytokines that can attract and polarize infiltrating leukocytes and therewith may have an antibody-independent role in sustaining inflammation.

We also observed increased frequencies of IL-6 and TNF\textsubscript{α+}+IL-6+ B cells upon \textit{in vitro} stimulation of peripheral B cells from newly diagnosed GCA patients (chapter 6) supporting a cytokine-mediated role of B cells in GCA. GC-treatment significantly reduces the frequency of these cytokine producing B cells. Long-term GC use gradually decreases total B cell numbers in the circulation as well (chapter 2). In treatment-free remission patients, we observed a similar leukocyte profile as in active disease with lowered T, B and NK cell numbers and increased monocytes and neutrophils (i.e. a myeloid shift). We therefore question if treatment-free patients are truly cured or still suffer from ongoing subclinical inflammation that could lead to late-stage complications. There is indeed evidence showing that GCA patients treated with either GC or tocilizumab can present with a normal CRP and ESR but still have persistent vessel wall inflammation\textsuperscript{46,47}. Interestingly, in these treatment-free remission patients ESR was negatively correlated with B cell numbers (chapter 2). B cells might therefore be important in preventing a disease flare or might aggravate disease by tracking to the site of inflammation.

In our proposed disease model, B cells play a role in disease pathogenesis by means of cytokine production and ATLO formation. In our view, B cells are involved in sustaining inflammation but are likely not involved in disease initiation. Nevertheless, as GCA is a chronic inflammatory disease with a high rate of relapse despite GC treatment, B cells
could be a new target of intervention in GCA. Alternatively, or perhaps simultaneously, B cells could be active in cleaning up the damaged vessel wall by proliferating and differentiating into antibody secreting cells that produce antibodies against vessel wall products\textsuperscript{36-38}. A study performing spatial transcriptomics of local B cells/ATLOs, potentially also over time, would be interesting to perform and could confirm the here proposed participation of B cells in disease pathogenesis.

**Could GCA and PMR patients benefit from B cell targeted treatment strategies?**

As the exact trigger(s) for disease initiation in GCA and PMR still remain(s) unclear, it is worthwhile to investigate why the inflammation in these diseases does not resolve. Despite some recent advances in the treatment of GCA, GC still represents the cornerstone in the treatment of GCA and PMR. GC repress the acute phase response and reduce inflammation markers CRP and ESR\textsuperscript{48}. However, recent evidence suggests that vessel wall inflammation persists in GCA patients while on GC-treatment and being in clinical remission\textsuperscript{46,47}. In addition, GC use is associated with multiple, sometimes serious, side effects that worsen with the duration of treatment\textsuperscript{49,50}. As it is the non-resolving inflammation that leads to chronic usage of GC and late-stage complications such as aortic aneurysms and dissections\textsuperscript{51,52}, treatment options interfering with chronic inflammation are highly needed.

Based on the evidence in this thesis, B cells are part of the inflammatory cascade in GCA (and possibly PMR), both via pro-inflammatory cytokine production and by ATLO mediated immune responses contributing to disease chronicity, and therefore it is interesting to consider B cell targeted treatment. So far there are only 2 case-reports of a B cell depletion therapy in GCA/PMR patients. One patient diagnosed with (C and LV) GCA and PMR received 1x 1000 mg rituximab (anti-CD20 monoclonal antibody in addition to 1x 100 mg methylprednisolone i.v.) and 1x 500 mg cyclophosphamide i.v. due to a second relapse while being treated with 15 mg prednisolone\textsuperscript{53}. After this combined therapy the patient reached clinical and biochemical (normalized CRP and ESR) remission. An FDG-PET-CT scan performed 4.5 months after rituximab-cyclophosphamide therapy showed also clear improvement of the FDG-vascular uptake. This patient, however, also received cyclophosphamide (i.e. B- and T-cell suppression therapy) making it unclear if the obtained improvement was solely a B cell depletion effect. The other case report was that of a GCA patient with concomitant neutropenia whose disease was inadequately controlled with GC\textsuperscript{54}. Unfortunately, there was no information of the therapeutic response provided in that case report. Recently, a phase 2 clinical trial (the BRIDGE-PMR study) showed modest beneficial effects of B cell depletion therapy with rituximab in PMR patients\textsuperscript{55}. This was a 21-week double-blind, placebo-controlled exploratory study of 47 patients with newly diagnosed PMR (n=38) or with relapsing disease on 7.5 mg per
Thesis summary and discussion

7
day or more of prednisolone (n=9). Patients were randomly assigned (1:1) to intravenous rituximab (1 × 1000 mg) or placebo in addition to 50 mg methylprednisolone, followed by a 17-week prednisolone taper to 0 mg. The primary outcome (glucocorticoid-free remission at week 21) was at the border of statistical significance (p=0.049) in favour of rituximab (11 (48%) of 23 patients in the rituximab group compared with five (21%) of 24 patients in the placebo group). In addition, the rituximab treated group also had a smaller proportion of patients on a GC maintenance dose (5 mg per day or less) and this group performed better on a clinical activity score. The results are encouraging and a larger and long-term randomized controlled trial is warranted.

There are lessons learned and more still to learn from other inflammatory diseases that have been treated with B cell depletion agents, even for diseases that are, like GCA and PMR, not considered to be B cell driven. B cell depletion therapy is for instance effective in the relapsing-remitting multiple sclerosis (RR-MS) and this effect seems to be largely caused by antibody-independent functions of B cells as antibody levels are mostly unaffected. By eliminating proinflammatory B cells, T cell responses and therewith disease relapses, are also reduced. Moreover, in MS and experimental autoimmune encephalomyelitis, B cell depletion therapy was shown to reduce IL-6 producing B cells to normal levels. This was accompanied by a reduction in autoreactive Th17 cells and disease severity. This strongly suggest that in MS, B cells contribute to T cell driven pathology by IL-6 production. Others also reported on a T cell decline in the cerebrospinal fluid after B cell depletion therapy in RR-MS. After rituximab treatment, it takes approximately six months for B cells to repopulate. In MS, the repopulated B cells produced normal levels of IL-6 while the percentage of memory cells did not change. This would suggest that the B cells themselves underwent a reset in cytokine production. Next to the effect of B cell depletion on T cells, B cells can also influence myeloid cell activation. In MS patients, a proinflammatory B cell subset, producing GM-CSF, IL-6 and TNF-α, was described to be increased. In a GM-CSF dependent manner, this B cell subset induced proinflammatory myeloid cell activation. B cell depletion therapy was accompanied by a reduction in proinflammatory myeloid cell responses in vivo and subsequently, this reduction also resulted in diminished proinflammatory T cell responses. Thus, B cell depletion therapy can have direct effects on T cell and myeloid cell responses but can also have indirect effects on T cells through myeloid cells.

These examples show that B cell depletion can be effective in diseases that are not primarily considered to be B cell driven. B cell depletion directly diminishes the antibody-independent functions of B cells and thereby interferes with the process of chronic inflammation. Given that cytokine producing B cells can influence T cells and macrophages, which remain the main cellular players in GCA and PMR, we provide here a rationale for B cell targeted therapy to be explored as a new treatment option, especially for the patients that have local B cell participation with ATLOs leading to non-
resolving inflammation. B cell targeted therapy could be given to patients at diagnosis as biopsies mostly reveal full blown inflammation already at this stage. However, due to the variability in B cells between patients, study results could also be variable. Therefore, it could be considered to give B cell targeted treatment to patients that show clear B cell infiltrated/ATLOs at the site of inflammation. As there is still an unmet need for treatment alternatives in GCA and PMR, and the B cell depleting rituximab is already an available disease modifying anti-rheumatic drug (DMARD), a trial with rituximab is highly awaited.

**Conclusion**

On the basis of our results, it is timely to give a place to B cells in the pathogenesis of GCA and PMR. We propose a model where B cells contribute to the immunopathology of GCA by cytokine production and as mediators of chronic inflammation by ATLO formation in the vessel wall. By the production of chemokines and cytokines that can attract and polarize myeloid and lymphoid cells towards pro-inflammatory and tissue destructive phenotypes, B cells fuel inflammation and add to the chronicity of disease. Our findings have laid a foundation for future research into the pathological role of B cells in these diseases and for considering the B cell as a new target of intervention in GCA and PMR.
References


