The role of B cells in giant cell arteritis and polymyalgia rheumatica
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Thesis Introduction
Chapter 1

The number of elderly individuals is steadily increasing mainly due to an increase in life expectancy\(^1\). This is mirrored by an increased burden on the health care systems as aging is associated with enhanced risk of infections, cardiovascular disease, cancer and autoimmunity\(^2\). Healthy aging is supported by living a healthy life, early diagnostics in case of health complaints and preventing heavy and long-term use of medications that come with considerable side-effects. The development of late onset autoinflammatory diseases is likely associated with aging of the immune system that culminates into a chronic low-grade inflammation, also known as inflammaging\(^3\). There are several autoinflammatory conditions related to aging. This thesis focusses on two aging related inflammatory diseases: giant cell arteritis (GCA) and polymyalgia rheumatica (PMR). GCA has a peak incidence at 72 years and is rarely diagnosed before the age of 50\(^4\). PMR is a closely related disease and is diagnosed in up to 60% of GCA patients\(^5\). Since the 1950’s, GCA and PMR have been treated with glucocorticoids (GC, prednisolone) which efficiently relieve disease symptoms and lower disease activity markers CRP and ESR\(^6\). However, GC treatment appears less effective at the level of the vessel wall\(^7\). In addition, long-term GC treatment, as required especially in GCA, is associated with considerable side effects, such as diabetes, hypertension, obesity, cataract, increased risk of infections and osteoporosis\(^8,9\) in these elderly patients. Efficacious, safe GC-sparing treatments in GCA and PMR are highly needed. Knowledge about the pathogenesis of these diseases is highly warranted to allow the identification of new targets for treatment and to decrease treatment associated morbidities.

**Giant Cell Arteritis and Polymyalgia Rheumatica**

Vasculitis is an inflammatory disorder of the blood vessels. Inflammation causes damage of the vessel wall that may eventually lead to ischemic injury of the end-tissue or organ. Based on the size of the affected arteries, vasculitides are divided into small-, medium- and large-sized vessel vasculitis. Giant cell arteritis (GCA) is one of the most common types of medium and large vessel vasculitis\(^10\). The highest prevalence is documented in northern European countries, with 20 or more GCA cases per 100,000 people over 50 years of age, and it is approximately 2 times more prevalent in woman compared to men.\(^4\) According to the involvement of different arteries in GCA, two forms can be distinguished; Cranial-GCA (C-GCA) and Large Vessel-GCA (LV-GCA) (see figure 1)\(^11\). C-GCA is the classical form of GCA, also known as temporal arteritis, as it often involves inflammation of the temporal artery. C-GCA mostly presents with typical symptoms like headache, jaw claudication, and visual disturbances\(^12\). If left untreated, C-GCA can lead to irreversible sight loss or stroke. LV-GCA is the systemic form, which affects the aorta and its main branches. The symptoms of LV-GCA are mostly non-specific (e.g., fever, malaise, anorexia and weight
loss), making the clinical diagnosis of LV-GCA more difficult than in C-GCA\textsuperscript{13}. In cranial and limb arteries, the inflammatory process can lead to stenosis and even occlusion of the blood vessel, while in the aorta, the inflammation can ultimately lead to aneurysm formation and aortic dissection. PMR is the second most common rheumatic disease in the elderly and is characterized by (teno)synovitis and bursitis causing pain and stiffness mainly in the shoulder- and hip girdle\textsuperscript{11}.

\textbf{Figure 1.} Graphical representation of the spectrum of diseases showing the overlap between Cranial giant cell arteritis (GCA), Large Vessel GCA and polymyalgia rheumatica (PMR). The percentages are a reflection of the overlap in the GCA PMR Senex (GPS) cohort of the UMCG.

Suspicion of GCA or PMR is based on clinical symptoms and supported by elevated acute phase response makers ESR and CRP. A temporal artery biopsy (TAB) is still often used to confirm a suspicion of GCA. Nevertheless, even on TAB, a diagnosis of C-GCA can be missed as the disease presents with “skip lesions”\textsuperscript{14}. Recent developments in medical imaging modalities have had a major impact on the diagnostic work-up in GCA and PMR. Both color duplex ultrasonography of the arterial wall, ultrasonography of the shoulders and hips, and the use of 18-fluorine fluorodeoxyglucose positron emission tomography combined with computed tomography (18F-FDG PET) have facilitated the recognition of GCA and PMR as conditions pertaining to the same spectrum of diseases\textsuperscript{15}. For instance, imaging revealed that, at diagnosis, up to 80\% of C-GCA patients can have (subclinical) large vessel involvement. In addition, approximately 33\% of PMR patients showed LV-GCA on imaging\textsuperscript{15}.
GC are still the mainstay of treatment in GCA and PMR and patients often require long-term GC use. In addition, disease relapses are common\textsuperscript{16} and necessitate high GC doses, which amplify treatment associated morbidity. Recent studies in GCA patients showed that, although the disease activity markers are strongly reduced by GC-treatment, vascular inflammation may persist\textsuperscript{17,18}. Alternative, GC-sparing, disease modifying anti-rheumatic drugs (DMARDs) are therefore needed for GCA and PMR patients. Some advances have been made with Methotrexate, a conventional synthetic DMARD, and more recently with the biological DMARD tocilizumab (Interleukin-6 receptor blocker) showing a GC-sparing effect and an increased percentage of GC-free remission patients as compared to GC monotherapy\textsuperscript{19,20}.

Pathogenesis

The pathogeneses of GCA and PMR are still incompletely understood. Knowledge on the pathogenesis of GCA is largely based on studies of the temporal artery as this is routinely taken for diagnostic purposes. As synovial biopsies are not routinely performed in PMR, much less is known about the immunopathology of PMR.

‘Healthy arteries’ are immunoprivileged sites with a clear distinction between the three different vessel wall layers, from outside to inside being adventitia, media and intima (see figure 2). At the adventitia-media border, tissue resident dendritic cells (DCs) are present and act as sentinel cells to capture antigens\textsuperscript{21}. After activation, DCs normally leave the peripheral tissue and migrate to adjacent lymphoid organs to induce immune responses. The most firmly established pathogenic model of GCA involves maturation and trapping of these activated resident DCs within the vessel wall rather than migration to lymphoid organs\textsuperscript{21}. DCs are generally activated through their Toll-like receptors (TLRs) that sense danger – or pathogen- associated molecular patterns (DAMPs, PAMPs). The trigger for activation of DCs in GCA is still unknown but both infectious agents\textsuperscript{22} and endogenous triggers\textsuperscript{10} have been proposed. There is, however, insufficient evidence to point towards a single responsible trigger. Activated DCs start producing various chemokines that initiate a pathogenic cascade by attracting CD4+ T-cells into the arterial wall\textsuperscript{10}. These cells are thought to enter the adventitia through the vasa vasorum and invade the vessel in an outward-inward direction\textsuperscript{23}. The CD4+ T-cells become polarized under the influence of local skewing cytokines towards T helper 1 (Th1) cells and T helper 17 (Th17) cells and start producing the corresponding cytokines, such as interferon g (IFNg) and interleukin-17 (IL-17), respectively\textsuperscript{10}. Other vascular resident cells, such as vascular smooth muscle cells, are hereby activated to produce chemokines, further attracting monocytes, CD4+ T cells, CD8+ T cells and B cells. The infiltrating monocytes become macrophages which organize into granulomas together with the
Figure 2. Vascular histology and schematic drawings of healthy temporal artery and aorta and a GCA-affected vessel. The tissues (upper panels) are stained with Hematoxylin and Eosin (HE) to reveal cell nuclei in blue. The inflamed temporal artery is characterized by expansion of the intima layer leading to stenosis and even occlusion of the lumen and a large number of infiltrating cells throughout the vessel wall. The structure of the aorta vessel wall is similar to that of the temporal artery but has a thicker layer of vascular smooth muscle cells in the media layer. The histology of GCA-affected aorta is assumed to be similar to the GCA-affected temporal artery. The drawings (lower panels) show the different layers in the healthy vessels and the infiltrated cell types in the inflamed vessel. The cellular composition of the infiltrates in the aorta remains to be investigated (?).
Chapter 1

**CD4+ T cells**\(^{24}\). Giant cells, the hallmark of giant cell arteritis, are large, multinucleated cells resulting from merged macrophages. This process establishes a pro-inflammatory cytokine milieu within the arterial wall. Moreover, local macrophages also produce tissue destructive proteins, such as matrix metalloproteinases (MMPs) that are able to break down the internal elastic lamina at the border between the media and intima layers, and hereby facilitate infiltration of the intima\(^{25}\). The artery responds with a remodeling process causing disorganization of the media, neo-intima formation and angiogenesis\(^{10}\). The formation of the neo-intima in cranial and limb arteries leads to intimal hyperplasia and even vessel occlusion which is responsible for the ischemic symptoms of these patients. In the aorta, breakdown of the media layer jeopardizes the vessel wall integrity eventually resulting in aortic aneurysm and/or dissection rather than occlusion\(^{26}\). Although the role of T cells and monocyte/macrophages has extensively been studied in GCA, the possible role of B cells in GCA pathology has long been neglected likely due to the absence of disease specific autoantibodies and given the scarce presence of B cells at the inflamed temporal artery\(^{27-30}\). However, more recent studies clearly document the presence of B cells at the site of vascular inflammation in C-GCA, albeit with a lower frequency than macrophages and T cells\(^{31,32}\). Little is known about the histopathology of PMR but one study reported infiltrates consisting of macrophages and T cells in the synovium, but no B cells\(^{33}\).

### B cells

B cells are lymphocytes pertaining to the adaptive immune system and are mostly known for their crucial role in humoral immunity. As B cells are the only cell type that can differentiate into antibody producing plasma cells, B cells have long been assessed for this function only. However, in the early-to-mid 1980s first evidence for other, antibody-independent functions of B cells were published\(^{34,35}\). These functions are antigen presentation and cytokine production (see figure 3).

B cells can act as antigen presenting cells after taking up an antigen through the B cell receptor, processing it and presenting it to CD4+ T-cells in the context of MHC Class II\(^{36}\). B cells can also produce an array of cytokines including the pro-inflammatory IL-6, IL-17, IFNγ, tumor necrosis factor α (TNFα), and granulocyte-macrophage colony-stimulating factor (GM-CSF), and the anti-inflammatory IL-10 and transforming growth factor β (TGF-β)\(^{36-38}\). Based on the cytokines that the B cells produce, they are identified as pro-inflammatory effector B cells or anti-inflammatory regulatory B cells. Effector B cells can polarize T cells but also myeloid cells such as macrophages\(^{36,39}\). Regulatory B cells have inhibitory effects on dendritic cells, macrophages, and T-cells and support the regulatory T cell population\(^{36-38}\). Of the cytokines that B cells can produce, IL-6, IL-17,
IFNγ, TNFα, and GM-CSF are all known players in the pathogenesis of GCA. B cells might thus contribute to disease pathology through cytokine production.

Figure 3. Antibody-dependent and antibody-independent functions of B cells. B cells can be activated through the Toll Like Receptors (TLR) or B cell receptor (BCR) and then can differentiate into antibody secreting plasma cells (PC), act as antigen presenting cells and produce cytokines.

**B cells in GCA and PMR**

Within the research field of GCA and PMR, B cells have so far not been a main focus of research and therefore little is known on their role in these diseases. However, the B cells have gained renewed interest after recent publications that suggest that B cells might play a more important role in GCA and PMR than initially believed.

Already in early studies, B cells and plasma cells were detected in the temporal artery of patients with C-GCA although they were clearly outnumbered by other cell types at this vascular site of inflammation. As in many chronic diseases, B cells were found to organize into artery tertiary lymphoid organs (ATLOs); being defined as B cell clusters with a follicular dendritic cell network and a germinal center, surrounded by T cells and high endothelial venules (HEV). HEV are specialized vessels that enable lymphocytes to migrate from the blood directly to the ATLO. Although most studies report the detection of B cells in the adventitia, the B cells organized in ATLOs were reported to...
be mainly detected in the medial layer of the inflamed temporal artery in GCA\textsuperscript{31}. The relevance of B cells and ATLOs for disease pathology and chronicity remains to be determined. Whether B cells are present in the inflamed aorta of LV-GCA is unknown.

There is some indirect evidence for a role of humoral immunity in GCA and PMR. Plasma cells were detected in inflamed TAB\textsuperscript{32,41} and increased IgG levels were reported in GCA patients\textsuperscript{42}. Moreover, patients suffering from permanent sight loss due to GCA were reported to more frequently have plasma cells present in the inflamed temporal artery\textsuperscript{41}. Given that humoral immunity is dependent on helper T cells, a study involving bilateral TAB of the same GCA patient, demonstrated identical TCR profiles on CD4+ T cells indicating that the immune response in GCA could be antigen-driven\textsuperscript{43}. The pathological role of antibody secreting cells is still unclear but several auto-antibodies have been described in GCA and PMR. These include anti-ferritin, anti-phospholipid and anti-nuclear lamin C antibodies\textsuperscript{44–46}. These are, however, not disease specific auto-antibodies and are mostly directed at arterial wall and cell components\textsuperscript{46}. Therefore, it is not clear if these auto-antibodies reflect a pathogenic mechanism or are secondary to tissue damage.

The peripheral blood is an easy accessible compartment to study systemic immunity as it likely mirrors inflammation at local sites and can therefore provide insight into possible disease mechanisms. In GCA and PMR, the distribution of B cell subsets in the peripheral blood is altered. Treatment-naïve GCA and PMR patients showed lowered numbers of circulating B cells compared to HC\textsuperscript{32,47,48}. More specifically, effector B cells (TNFα+IL-10-) were lowered in these treatment-naïve patients while regulatory B cells (TNFα-IL-10+) were unaltered\textsuperscript{32}. The number of peripheral blood B cells in a pooled GCA and PMR population inversely correlated with ESR\textsuperscript{32}. Another study reported a positive correlation between CRP and numbers of peripheral blood B cells in patients with PMR\textsuperscript{47}. Upon GC-induced remission, effector B cell numbers increased in the circulation without signs of compensatory hyperproliferation or enhanced bone marrow production. These findings led to the hypothesis that this increase of peripheral B cell numbers is caused by B cells returning to the circulation from peripheral sites (inflamed blood vessels in GCA, synovium or lymphoid tissue in PMR) upon GC-induced remission\textsuperscript{32,47,48}. Alternatively, B cells could be released from the marginal pool. The increase of B cells after treatment was also observed in PMR patients treated with tocilizumab monotherapy, suggesting a link to disease itself rather than a GC-related effect\textsuperscript{47}. The returning effector B cells showed an enhanced potency to produce the pro-inflammatory cytokine IL-6, indicating that B cells can become activated and contribute to the enhanced IL-6 response in GCA and PMR patients\textsuperscript{32}. 
Aims and outline of this thesis

Despite the recent evidence of an altered B cell homeostasis in GCA and PMR, there is still a knowledge gap regarding the role of B cells in these diseases. The aim of this thesis is to determine if B cells play an antibody-independent role in the disease pathology of GCA and the related disease PMR. To this end, we study B cells at both the local site of vascular inflammation (temporal artery and aorta) as well as the peripheral blood of C-GCA, LV-GCA and PMR patients. We first analyze B cell dynamics in the peripheral blood and study B cell presence and organization in the inflamed arteries of GCA patients. We then focus on circulating B cell subsets in GCA and PMR and assess which factors are involved in B cell trafficking by investigating chemokines and chemokine receptors both locally and in the peripheral blood. Lastly, we investigate the cytokine production of arterial B cells in GCA and circulating B cells in GCA and PMR and assess the potential of GCA B cell-conditioned medium on macrophage polarization in vitro.

In chapter 2 we comprehensively analyze leukocyte counts in the peripheral blood of GCA and PMR patients to determine whether they can be used as cellular makers of inflammation. Counts of six leukocyte subsets are documented in a well-defined and prospectively followed cohort of newly diagnosed GCA and PMR patients as compared to healthy controls. We analyze leukocyte subsets before and during GC treatment and also in GC-free remission.

In chapter 3 the presence, organization and localization of B cells in the inflamed temporal artery of GCA patients is addressed. The data comprise our response to a study reporting that B cell clusters or ATLOs are exclusively detected in the media layer of the temporal artery.

Chapter 4 provides a histopathological study on B cells in LV-GCA aorta tissue obtained from patients undergoing surgery due to an aortic aneurysm or dissection. We compare B cell infiltration and organization in aortic tissues from GCA patients to aortic tissues from patients with atherosclerosis. We assess the location of B cells in the inflamed aorta and their degree of organization by studying germinal center and proliferation markers. In addition, we document the presence of plasma cells and determine their IgM and IgG expression.

Chapter 5 focuses on chemotactic mechanisms responsible for B cell trafficking in GCA and PMR. We assess serum chemokine expression in treatment-naïve and GC-induced remission patients and investigate chemokine receptor expression on circulating B cells subsets. Next, we determine if the altered chemokines with the corresponding chemokine receptors are also expressed at the B cell areas in temporal artery and aorta. We also compare the chemokine-chemokine receptor expression by B cells between C-GCA and LV-GCA patients.
In **chapter 6** we aim to assess if cytokine producing B cells are active players in the vasculopathy of GCA. B cell cytokine expression is studied in the temporal artery and aorta tissues of GCA patients as well as in the aorta of patients with atherosclerosis. We analyzed both pro-inflammatory and anti-inflammatory cytokines as it is yet unknown if B cells and ATLOs are protective or pathogenic. Next to the arterial B cells, we also investigate the cytokine producing capacity of B cells in the circulation of treatment-naïve and GC-treated GCA and PMR patients. Lastly, as B cell derived cytokines may influence macrophage phenotype and function, we assess the *in vitro* effect of GCA and HC derived B cell conditioned medium on macrophages.

Finally, in **chapter 7**, the obtained results are summarized and discussed. Based on our findings we provide an outlook for future research.
References


