SUMMARY AND DISCUSSION
Summary

Aging of the immune system may contribute to the development of aging-associated autoimmune diseases, such as giant cell arteritis, polymyalgia rheumatica and rheumatoid arthritis. The aim of this thesis was to identify aging-dependent changes of the adaptive immune system that promote autoimmunity in the elderly. The first chapters of this thesis describe changes of the adaptive immune system occurring during healthy aging (chapters 2 to 6). Furthermore, we link maintenance of particular T and B cell populations to risk factors for aging-associated autoimmune diseases and development of circulating autoantibodies (chapters 2 and 6). The final chapters of this thesis focus on changes of the adaptive immune system in patients with aging-associated autoimmune diseases, i.e. giant cell arteritis and polymyalgia rheumatica (chapters 7 to 9). Furthermore, the diagnostic potential of cellular and soluble immune parameters is explored in the latter two diseases (chapters 8 and 9).
Aging of the adaptive immune system

The adaptive immune system, with T and B cells as key representatives, is characterized by two fundamental principles: antigen-specificity and the development of immunological memory. Whereas B cells recognize soluble antigens via their B cell receptors, T cells are equipped with T cell receptors (TCR) recognizing antigens presented by major histocompatibility complex (MHC) molecules on antigen presenting cells. As every T cell and B cell expresses antigen receptors specific to one particular antigen, the ability of the adaptive immune system to respond to novel antigens is highly dependent on the size and diversity of the antigen receptor repertoires within the naive T and B cell pools. Following antigenic recognition, T and B cells become activated and start to proliferate. Eventually, part of these cells will differentiate into memory T and B cells. These memory cells directly provide the effector functions of the adaptive immune system (e.g. cytokine production, cytotoxicity) or differentiate even further (i.e. antibody-secreting plasma cells). Development of immunological memory allows the adaptive immune system to respond faster and more efficient to subsequent challenges with the same antigens.

Impact of aging on the T cell receptor repertoire

T cells are critical players in the immune system, as CD4+ T cells provide help to other immune cells, while CD8+ T cells kill virally-infected host cells and tumour cells. A large and diverse naive TCR repertoire is required in order to develop T cell immunity against a wide array of microbial and tumour antigens [1]. In chapter 4, we combined flow cytometric TCR analysis with economic statistics and demonstrated that aging is associated with skewing of the TCR repertoire of CD4+ and CD8+ T cells. In addition, we found that naive T cells demonstrate the broadest TCR repertoire, whereas progressive repertoire skewing was observed among central memory, effector memory and terminally differentiated T cells. Furthermore, we confirmed that cytomegalovirus (CMV) skews the TCR repertoire in otherwise healthy individuals. Two studies applying TCR sequencing have recently shown that the actual number of T cell clones, i.e. TCR diversity, is decreased in the elderly [2,3]. One of these studies, however, measured TCR diversity in peripheral blood mononuclear cell fractions, rather than within well-defined populations of T cells and did not account for CMV serostatus [2]. Therefore, the results from this study may have been influenced not only by alterations in the cellular composition of the peripheral blood mononuclear cell fractions, but also by differences in CMV serostatus between young and old individuals. In contrast, Qi et al. performed their TCR sequencing on sorted naive CD4+ and
CD8+ T cell fractions of CMV seronegative individuals [3]. These authors showed that clonal diversity within these T cell fractions declines with age, whereas the size of distinct clones becomes more unequal (i.e. skewed distribution). Taken together, both aging and CMV infection may compromise the diversity of TCR repertoire. These findings are relevant, as contractions of the TCR repertoire have been associated with poor responses to vaccination, viral infections and cancer [4-6].

### Decline of thymic output with aging

Naive CD4+ and CD8+ T cells show the broadest TCR repertoire (chapter 4). The production of these cells, however, dramatically declines with aging [7], as adipocytes progressively replace thymic epithelial cells in the thymus [8]. This process of thymic involution alters the daily thymic output from 16 million cells per day in young subjects to less than 1 million cells per day in aged subjects [9].

Early T cell precursors are created in the bone marrow. Unlike other immune cells, T cells require maturation in another primary lymphoid organ, i.e. the thymus [10]. In the thymus, T cells undergo recombination of TCR genes, which eventually results in expression of a unique TCR. Eventually, precursor T cells with functional TCR proceed their maturation, whereas T cells with non-functioning TCR go into apoptosis. This process of positive selection requires the newly-created TCR to recognize self-peptide/MHC complexes in the thymus. Subsequently, the positively selected, precursor T cells will undergo negative selection to prevent the maturation of T cells with high affinity for self-peptide/MHC complexes. Eventually, only naive T cells carrying a TCR with low affinity for self-peptide/MHC complexes are allowed to enter the circulation. As mentioned further on, aging-associated disruption of the thymic selection process could be involved in the development of aging-associated autoimmune diseases.

### Peripheral maintenance of naive CD4+ T cells in the elderly

As thymic output declines, homeostatic maintenance of already existing T cells is critical to preserve the T cell pool in aged individuals [7]. An estimated 100 million T cells per day are currently thought to be produced in the periphery of healthy adults [9]. Animal studies have shown that peripheral maintenance of naive T cells requires low affinity recognition of self-peptide/MHC complexes and stimulation by interleukin-7 (IL-7) [11]. An important role for IL-7 in the homeostatic maintenance of naive T cells in humans is supported by findings in a phase I trial with recombinant IL-7 [12]. In chapter 3, we provide evidence that naive CD4+ T cells in elderly humans become responsive to another homeostatic cytokine, i.e. interleukin-2 (IL-2). These IL-2 responsive cells are characterized by dim expression of the IL-2 receptor α chain (IL-2Rα; CD25), and their numbers expand substantially with aging. Interestingly, these CD45RA+CD25dim CD4+ T cells develop upon low affinity TCR engagement in secondary lymphoid
tissues. These findings are remarkable, as IL-2 was considered important for the homeostasis of memory T cells and regulatory T cells only [11]. The enhanced sensitivity to IL-2 may be an important adaptation of naive CD4+ T cells to the changing cytokine milieu in the elderly, since serum levels of IL-2 remain stable with aging, whereas IL-7 levels decline [13-15]. Interestingly, no increase in IL-2 responsive CD8+ T cells was observed in aged subjects, thereby implying that the maintenance of naive CD4+ and CD8+ T cells is differentially regulated. Overall, our findings indicate that TCR engagement and homeostatic cytokines jointly promote the maintenance of a broad, naive T cell repertoire in the elderly (Figure 1).

Both heritable and non-heritable factors appear to influence the maintenance of distinct population of T cells. Recently, Brodin et al. showed that inter-individual variation in naive CD4+ T cell numbers is mostly explained by heritable factors, whereas that of naive CD8+ T cells is mostly determined by non-heritable factors [16]. In chapter 2, we identify two heritable factors that may partly explain inter-individual variation in naive CD4+ T cell numbers. The first factor is gender. In accordance with prior studies, we observed that females maintain higher numbers of CD4+ T cells than males [17,18]. We demonstrate that this difference results from higher numbers of naive CD4+ T cells in women (chapter 2). Hormonal effects on the proliferation of naive T cells may contribute to this gender difference.

Figure 1. Schematic representation of naive CD4+ T cell maintenance in humans of young and old age. At young age thymic output is substantial and contributes heavily to maintenance of the naive CD4+ T cell pool. In contrast, thymic output is limited at old age and peripheral maintenance mechanisms become more important to preserve the naive CD4+ T cell pool. Interleukin-2 contributes to the homeostasis of naive CD4+ T cells in old but not young individuals.
The second factor is HLA-DR4 carriage. We observed that naive CD4+ T cells were substantially better maintained in HLA-DR4 positive individuals than in HLA-DR4 negative individuals. This finding is interesting, as HLA-DR4 positivity has been associated with premature aging of the immune system, i.e. shortening of telomeres in immune cells [21]. Although the exact relation between HLA-DR4 positivity and enhanced naive CD4+ T cell maintenance remains to be elucidated, it might be possible that HLA-DR4 promotes the homeostatic proliferation of naive CD4+ T cells recognizing self-peptides presented by HLA-DR4. This would also explain the observation of telomere shortening in CD4+ T cells of HLA-DR4 positive individuals [21].

CMV infection, which represents a non-heritable factor shaping the memory T cell compartment of humans, had no effect on the maintenance of naive CD8+ T cells (chapter 2). In contrast, latent CMV infection was associated with slightly decreased numbers of naive CD4+ T cells in aged individuals but not in young individuals. Recently, Wertheimer et al. also observed that CMV only affects naive CD4+ T cell numbers in aged subjects [22]. Although the impact of CMV on the naive CD4+ T cell compartment of aged subjects may seem subtle, a recent study suggests that it is associated with declining CD4+ T cell responses to influenza vaccination in the elderly [23].

**Effect of aging on balance between T helper cells and regulatory T cells**

Although the concept of ‘inflammaging and anti-inflammaging’ has been mostly studied in the context of the innate immune system [24,25], a similar paradigm may be present in the memory T cell compartment of humans. The memory CD4+ T cell compartment encompasses distinct populations of pro-inflammatory T helper (Th) cells. In chapter 5, we show that Th17 cells are decreased in the circulation of aged humans, whereas Th1 and Th2 cells are largely retained. In contrast, we found that memory regulatory T (Treg) cells are increased in the elderly. Consequently, the overall balance between Th cells and memory Treg cells shifted towards the latter in healthy, elderly subjects. The balance between Th cells and memory Treg cells is important, as these cell populations share similar homing receptors (chapter 5) and likely meet *in vivo* during immune responses [26].

An aging-associated increase in Treg cells, however, may come at the expense of immunity to microbes and tumour cells, as evidenced by animal studies [27-29]. One human study has shown that Treg cells compromise antimicrobial immunity in the skin of aged subjects [30]. We extended this finding in chapter 5, by showing that aged subjects with the most pronounced increase in Treg cells demonstrated poor responses to influenza vaccination. Thus, the balance between pro-inflammatory and anti-inflammatory memory CD4+ T cells is delicate in the elderly.
Aging of the CD8+ T cell compartment

CD8+ T cells are critical for immunity against viruses and cancer. In *chapter 2*, we show that memory CD8+ T cell populations are well-retained with age, whereas naive CD8+ T cells dramatically decline. The decline in naive CD8+ T cells may result from insufficient adaptation to the changing cytokine milieu, as naive CD8+ T cells of elderly subjects are not responsive to IL-2 (*chapter 3*). Alternatively, other studies indicate that naive CD8+ T cells display lower thresholds for acquiring a memory phenotype than naive CD4+ T cells, and are therefore recruited more extensively towards the memory compartment. Indeed, we observed that a single shot of DTaP-IPV vaccine (diphtheria, tetanus, acellular pertussis, inactivated poliovirus) results in a more substantial decrease of naive CD8+ T cells than naive CD4+ T cells (*chapter 4*).

Earlier studies suggested that naive CD8+ T cells decline with aging due to progressive occupation of ‘immunological space’ by memory CD8+ T cells. This notion, however, was solely based on proportional data. Absolute numbers of memory CD8+ T cell populations did not increase with age in our study (*chapter 2*). Our findings are in agreement with those from two recent studies [22,31]. As the increased proportions of memory CD8+ T cells in aged subjects reflect an absolute decrease in naive CD8+ T cells rather than a true increase of memory CD8+ T cells, the original ‘immunological space’ theory seems incorrect. It is also difficult to understand how memory CD8+ T cells would compete with naive CD8+ T cell for ‘immunological space’, since these cells thrive in different niches and respond to different homeostatic cytokines [11]. Instead, it is more likely that different clonal specificities of memory CD8+ T cells compete for ‘immunological space’ amongst each other. As maintenance of T cell memory is an active process requiring constant proliferation of short-lived memory cells [32], substantial outgrowth of memory CD8+ T cells directed to one pathogen could compromise memory to other pathogens. A particular pathogen that is associated with substantial clonal expansions is CMV [33]. In accordance with prior studies, we observed that CMV infection is associated with a strong increase in memory CD8+ T cells and profound skewing of the TCR repertoire among CD8+ T cells (*chapters 2 and 4*). Interestingly, CMV infection compromises long-term CD8+ T cell immunity to Epstein-Barr virus [34]. It remains to be elucidated whether CMV also affects immunity to other viruses, such as varicella zoster virus and influenza virus.

Impact of aging on cytokine production in B cells

Accumulating evidence indicates that B cells are not only precursors for antibody-secreting cells, but also actively influence immune responses by providing T cell help and secreting cytokines [35]. Previously, B cells have been divided into effector B cells producing pro-inflammatory cytokines, such as TNF-α and IL-6, and regulatory B cells producing anti-inflammatory cytokines, e.g. IL-10 and TGF-β.
It has been postulated that these B cell populations are truly distinct B cell lineages, analogous to Th cells and Treg cells in the CD4+ T cell compartment [35]. However, recent data indicate that production of pro-inflammatory and anti-inflammatory cytokines represents a transient functional program in B cells rather than a true lineage program [36].

Only few studies have assessed the impact of aging on the production of cytokines by B cells. Frasca et al. have demonstrated that B cells of aged subjects contain more mRNA transcripts for TNF-α than those from young subjects [37]. Duggal et al. have reported that transitional B cells of aged subjects less efficiently differentiate into IL-10 producing B cells than those from young subjects [38]. In chapter 6, we studied the actual number of TNF-α and IL-10 producing B cells in the circulation of aged humans by shortly stimulating B cells with phorbol 12-myristate 13-acetate (PMA) and calcium ionophore. We observed that TNF-α producing B cells are retained in the circulation of aged subjects, whereas IL-10 producing B cells are decreased. Recently, Khoder et al. found that transitional B cells and unswitched memory B cells show the highest potential to differentiate into IL-10 producing cells [39]. Using our direct stimulation approach, we observed that IL-10 producing B cells are primarily found among the unswitched, and to some extent, switched memory B cell compartments (chapters 6 and 7). TNF-α producing cells were observed among all memory B cell populations. Our findings therefore suggest that cytokine production in human B cells primarily depends on the differentiation stage of these cells.

**Immune-aging and autoimmunity**

Aging is associated with development of autoimmunity [25]. Giant cell arteritis (GCA) and polymyalgia rheumatica (PMR) solely develop in individuals of 50 years and older, whereas rheumatoid arthritis (RA) shows a peak incidence around the age of 60 [40]. Furthermore, the prevalence of circulating auto-antibodies is increased in otherwise healthy, elderly subjects (chapter 6). Besides cells of the innate immune system, CD4+ T cells and B cells are critical players in development of aging-associated autoimmunity.

**CD4+ T cell maintenance and autoimmunity**

GCA and RA, and to some extent PMR, are considered CD4+ T cell driven diseases [41,42]. Pro-inflammatory T helper cells are increased in these diseases and found at local sites of inflammation. The importance of CD4+ T cells in these aging-associated autoimmune diseases is further suggested by their association with the MHC class II molecule, HLA-DR4. This disease association can be attributed to amino acids in the peptide binding groove of HLA-DR4, which is involved in antigen presentation to CD4+ T cells [43-45]. The importance of CD4+ T cells in aging-associated autoimmune disease is intriguing, as these diseases develop long after thymic output has declined. A key question therefore is whether these
autoreactive CD4+ T cells are created by the aged thymus, or already early in life as are most circulating CD4+ T cells in aged subjects. If autoreactive CD4+ T cells are already produced in early life, mechanisms promoting the long-term maintenance of these T cells may be critical for development of aging-associated autoimmune diseases. This scenario is supported by the observation that the T cell pool of aged individuals is maintained via peripheral proliferation (approximately 100 million cells per day) rather than thymic output (estimated 1 million cells per day) [32]. Furthermore, we show in chapter 2 that important risk factors for aging-associated autoimmune diseases, i.e. HLA-DR4 positivity and female gender, are indeed associated with enhanced maintenance of the naive CD4+ T cell repertoire. Although no increase in naive CD4+ T cells was observed in GCA and PMR patients (chapter 8), we recently observed increased numbers of naive CD4+ T cells in RA patients [46]. Furthermore, patients with seropositive arthralgia, who are at risk for development of RA, also tended to show higher numbers of circulating, naive CD4+ T cells when compared to healthy controls [46]. Animal studies by Busser et al. also support the notion that a diverse T cell repertoire is required to develop autoimmunity [47]. Thus, maintenance of a broad and diverse T cell repertoire in the elderly may be beneficial for immunity against microbes and tumour cells, but at the same time increase vulnerability for development of autoimmunity.

Alternatively, it is possible that aging-associated autoimmunity develops as the aged thymus produces highly self-reactive CD4+ T cells due to defects in negative selection. Indeed, central tolerance is disturbed in the thymus of aged mice, as thymic epithelial cells show less autoimmune regulator (AIRE) gene expression with aging [48]. Consequently, the negative selection of autoreactive T cells is disturbed in the thymus of aged animals. Although direct translation of these findings to humans is difficult, it is likely that the thymus of aged humans also exhibits defects in central tolerance, as the gradual replacement of thymic epithelial cells by adipocytes will limit the negative selection process of precursor T cells.

B cell maintenance and autoimmunity

Our findings in chapter 6 indicate that the balance between pro-inflammatory B cells and anti-inflammatory B cells shifts towards the first. Pro-inflammatory B cells producing TNF-α were retained in aged subjects while B cells producing IL-10 were decreased. In chapter 7, we show that these pro-inflammatory B cells may contribute to the immunopathology of GCA and PMR via enhanced production of IL-6. As IL-10 producing B cells may exert regulatory functions, their decrease could render aged subjects at risk for autoimmunity. Indeed, we observed an association between declining numbers of IL-10 producing B cells and development of anti-nuclear antibodies in elderly subjects. However, IL-10 producing B cells were remarkably well retained in aged subjects that were
seropositive for rheumatoid factors. The latter finding is interesting, as IL-10 is known to promote rheumatoid factor production in B cells of RA patients [49]. Furthermore, IL-10 producing B cells are increased in the circulation of RA patients [50]. These findings could indicate that IL-10 producing B cells promote the development of rheumatoid factors in the elderly. Thus, IL-10 producing B cells may have a dual role in aging-associated autoimmunity.

**Giant cell arteritis and polymyalgia rheumatica**

**Pathogenesis**

GCA and PMR are common, aging-associated autoimmune diseases [40]. GCA is a systemic vasculitis affecting large and medium-sized arteries [42], whereas PMR is characterized by mild synovitis, enthesitis and bursitis [51]. Interestingly, these diseases frequently co-occur [42].

The current pathogenic model for GCA is primarily focused on dendritic cells (DCs), T cells and monocytes/macrophages [52], as shown in Figure 2. DCs residing in the arterial wall are thought to initiate vascular inflammation in GCA patients. These resident DCs express distinct Toll-like receptors in different arteries, which

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**Figure 2. Schematic model of the pathogenesis of giant cell arteritis.** The inflammatory response in the arterial wall is initiated when Toll-like receptors (TLRs) on resident dendritic cells are activated by TLR ligands. Activated dendritic cells attract Th1 and Th17 cells, which cause vascular inflammation. Eventually, monocytes enter the vascular wall and differentiate into macrophages promoting vascular inflammation via secretion of cytokines and vascular damage via secretion of matrix metalloproteinases (MMPs).
could explain why certain vascular beds are affected in some GCA patients but spared in others [53]. Vascular DCs may become activated upon exposure to microbial (or perhaps endogenous) ligands for Toll-like receptors. Subsequently, these DCs attract Th1 and Th17 cells which in turn promote vascular inflammation and attract monocytes. Various studies have shown that Th1 cells and Th17 cells are abundant in the inflamed arteries of GCA patients [54,55]. In addition, Th1 and Th17 cells are also expanded in the circulation of GCA patients [54,56]. In accordance with the notion that CD4+ T cells are key players in GCA, we observed enhanced activation and proliferation of memory CD4+ T cells in GCA (chapter 8). Once in the vascular wall, monocytes differentiate into macrophages that further promote inflammation and vascular damage [57-59]. In essence, less is known about immunopathology of PMR. Samson et al. observed increased numbers of Th1 and Th17 cells in the circulation of PMR patients. However, we observed little activation and proliferation of circulating CD4+ T cells in PMR patients (chapter 8). It might be possible though that activated CD4+ T cells migrate extensively from the circulation towards inflamed joints of PMR patients (Figure 3). Even less is known about the local inflammatory process in patients with PMR. Although Meliconi et al. have shown that T cells and macrophages predominate in inflamed synovium of PMR patients [60], the functional properties of these infiltrating cells remain to be elucidated.

Figure 3. Paired peripheral blood and synovial fluid analysis of a polymyalgia rheumatica patient. (A) FDG-PET-CT scan showing enhanced glucose uptake around the shoulders, hips and knees of the patient. (B) No clear signs of osteoarthritis on knee X-ray of the patient. (C) Analysis of CD45RO and CCR7 defined CD4+ T cell subsets in peripheral blood and synovial fluid of the patient. CD45RO+CCR7- effector memory CD4+ T cells predominated in the synovial fluid.
In chapter 7, we extend the pathogenic models for GCA and PMR by showing that pro-inflammatory B cells, identified by TNF-α production, are redistributed during active disease and return to the circulation upon corticosteroid-induced remission. As we confirmed that B cells are not pre-dominant cells in temporal arteries of GCA patients [57], and others have observed few B cells in synovial tissue of PMR patients [60], we presume that B cells are redistributed towards lymphoid organs during active disease. Indeed, profound activation of B cells in secondary lymphoid organs of GCA and PMR patients is also suggested by the increased prevalence of various types of auto-antibodies in sera of these patients [61,62]. Furthermore, enhanced production of IL-6 by pro-inflammatory B cells in GCA and PMR patients suggests interaction between B cells and T cells in secondary lymphoid organs, as Barr et al. have shown that B cells require T cell help in order to produce IL-6 [63]. Interestingly, clinical studies with B cell depletion therapy and in vitro experiments have shown that B cells are critical for Th17 cell expansion [64]. Therefore, we propose that B cells might promote the expansion of Th17 cells in secondary lymphoid organs of GCA and PMR patients (Figure 4).

**Figure 4. Proposed role of B cells in giant cell arteritis and polymyalgia rheumatica.** Interaction between B cells and T cells in secondary lymphoid tissues promotes IL-6 production in B cells and induction and expansion of Th17 cells.
Little is known about the role of other immune cells in GCA and PMR. Recently, Nadkarni et al. reported that neutrophils may play a role in GCA [65]. Indeed, neutrophils are sometimes observed in arterial walls of GCA patients [66]. Absence of neutrophils in most temporal artery biopsies might indicate that most patients are diagnosed during the chronic phase of GCA, rather than the acute phase. In contrast to CD4+ T cells, CD8+ T cells are likely of little importance in GCA and PMR. CD8+ T cells are hardly present in inflamed temporal arteries of GCA patients [54], and we observed no substantial modulation in circulating CD8+ T cells subsets (chapter 8).

**Symptoms and diagnosis**

GCA patients with inflammation of cranial arteries may present with typical symptoms such as headache, blindness, jaw claudication and stroke [42]. In contrast, GCA with involvement of systemic arteries (i.e. aortic arch, subclavian arteries) primarily show general symptoms of systemic inflammation, such as malaise, weight loss and fever. PMR patients typically present with pain and stiffness of shoulders, neck, back and hips [51]. A diagnosis of GCA or PMR is suspected, if patients demonstrate symptoms suggestive of GCA or PMR and elevations in systemic inflammatory markers.

The erythrocyte sedimentation rate (ESR) and serum levels of C-reactive protein (CRP) are increased in 90% of biopsy-proven GCA patients, whereas both these inflammatory markers remain normal in 4% of biopsy-proven patients [67]. Notably, during follow-up of corticosteroid treated patients the sensitivity of the ESR and CRP are further compromised [68]. Novel biomarkers for active GCA and PMR are therefore needed. In chapters 8 and 9 we explored the utility of T cells and a large panel of serum markers for diagnosing active GCA and PMR. Although we observed significant changes of T cell differentiation subsets, activation markers and proliferation markers in GCA and PMR patients, these perhaps biologically relevant modulations provided limited diagnostic accuracy for diagnosing active GCA and PMR. Among a panel of 26 serum markers, all of which related to immune cells involved in the pathogenesis of GCA and PMR, we identified serum IL-6 and B cell activating factor (BAFF; also termed Blys) as promising markers for active GCA and PMR. Larger longitudinal studies are required to determine the diagnostic value of these markers for clinical practice. In addition, measuring IL-6 and BAFF in inflammatory control subjects, such as patients with infections, will be required to obtain insight into their specificity.

So far, temporal artery biopsy has remained the gold standard test for diagnosing GCA. Recent studies have described the spectrum of vascular inflammation that might be observed in GCA patients [66]. Imaging techniques, i.e. ultrasound and FDG-PET-CT scanning, have become an important tool in the diagnostic workup of suspected GCA patients [69]. In clinical trials, imaging techniques are already regarded equally important as a temporal artery biopsy.
Ultrasound of temporal, carotid, axillary and subclavian arteries is a promising technique to detect active GCA. Ultrasound has a high sensitivity and specificity in the hands of experienced investigators [70]. FDG-PET-CT scanning may reveal extensive inflammation of large, systemic arteries in 83% of temporal artery biopsy-proven GCA patients [71]. Furthermore, FDG-PET-CT scanning is the test of choice in patients with systemic symptoms suspected of having inflammation of the aorta and its direct branches. In addition, ultrasound and FDG-PET-CT scanning may also aid the diagnosis of PMR [51]. It remains an important challenge to identify biomarkers separating PMR patients with and without large vessel vasculitis.

**Treatment and prognosis**

Corticosteroids currently remain the cornerstone of treatment in GCA and PMR, despite efforts to identify additional therapies. A meta-analysis of 10 randomised controlled trials has shown that addition of low dose methotrexate to the corticosteroid treatment only slightly reduces relapses and cumulative corticosteroid doses in GCA patients [72]. In addition, mixed efficacy of methotrexate has been observed in PMR patients [51]. Randomized controlled trials have shown that TNF-α blocking therapy is not effective in GCA and PMR patients [73,74]. Currently, trials with IL-6 receptor blocking therapy, IL-1R blocking therapy and anti-CD80/CD86 therapy are in progress. Our findings in chapter 7 would also justify studying B cell depletion therapy in GCA and PMR. Most PMR patients are withdrawn from corticosteroids after 1-2 years of therapy [51]. In contrast, only 50% of GCA patients can be fully tapered after 2 years of treatment [75]. Identifying prognostic biomarkers will be important to develop therapeutic strategies based on relapse risks. An important long-term complication in GCA patients is the development of aortic aneurysms [76]. Further studies on the pathogenesis, prevention and clinical management of these aneurysms are highly needed.

**Concluding remarks**

Development of autoimmunity in the elderly is intriguing, as it implies that aging impairs the control of autoreactive immune cells. Understanding the mechanisms facilitating the generation and maintenance of autoreactive immune cells may therefore greatly help to determine how aging-associated autoimmune diseases eventually develop. This thesis describes how certain T and B cell populations modulate with age, whereas others remain stable. We show that preservation or modulation of certain T and B cell populations in the elderly may be linked to risk factors for autoimmunity (i.e. HLA-DR4, female gender) or development of autoantibodies (i.e. rheumatoid factors, anti-nuclear antibodies), respectively. Furthermore, this thesis offers mechanistic insight into the maintenance of the naive CD4+ T cell pool by showing that low affinity TCR engagement and IL-2
are involved in this process. Studying both young and old healthy individuals enabled us to better understand the development of aging-associated autoimmune diseases. For instance, we observed that pro-inflammatory B cells are maintained until high age and that these B cells likely contribute to the immunopathology of GCA and PMR. A key challenge for the future will be to longitudinally study elderly individuals before and after onset of aging-associated autoimmune diseases. Finally, we describe promising biomarkers for disease activity in GCA and PMR patients, which require further validation in large-scale, longitudinal studies.

References


Summary and discussion


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