CHAPTER 1

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Introduction

ANCA-associated vasculitis

Vasculitis is an inflammatory process of blood vessels, histopathologically characterized by vessel wall destruction and occlusion of the vascular lumen. Vasculitis is classified based on the site of the blood vessels involved, the histopathology of the lesions, and clinical findings [1]. Within the group of small vessel vasculitides, Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS) and renal-limited vasculitis (RLV) are acknowledged to be associated with anti-neutrophil cytoplasmic autoantibodies (ANCA). They are designated as ANCA-associated vasculitis (AAV). ANCA are predominantly IgG class autoantibodies directed against constituents of granules of neutrophils and lysosomes of monocytes. By indirect immunofluorescence (IIF) on ethanol-fixed neutrophils, two fluorescence patterns of ANCA are distinguished, the cytoplasmic staining pattern (cANCA) and the perinuclear staining pattern (pANCA). Most patients with a cANCA pattern obtained by IIF have ANCA directed against proteinase-3 (PR3), as determined by antigen-specific ELISA. Patients with pANCA have ANCA directed against a variety of antigens, but in the primary small vessel vasculitides, the target antigen is almost invariably myeloperoxidase (MPO). The combinations of a cANCA pattern by IIF with PR3-ANCA by ELISA and a pANCA pattern by IIF with MPO-ANCA by ELISA are very specific for AAV [2].

ANCA-associated vasculitis and activation of the complement system

The pathogenesis of AAV has not yet been fully elucidated. There is increasing clinical, in vitro and in vivo experimental evidence for a pathogenic role of ANCA in the pathogenesis of ANCA-associated vasculitis. For example, in vitro studies have shown that ANCA are able to stimulate neutrophils which are primed by cytokines, like TNFα, to degranulate their granules and induce a respiratory burst with the release of toxic oxygen radicals and lysosomal enzymes, leading to tissue damage [3-6]. In vivo experimental studies demonstrate that passive transfer of anti-MPO IgG into mice can induce pauci-immune focal necrotizing and crescentic glomerulonephritis whereas some mice also develop systemic small vessel vasculitis [7]. Both the innate and the adaptive immune system
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are involved in the pathogenesis of AAV but the precise pathway along which lesions develop is still open for discussion.

AAV is characterized by pauci-immune necrotizing crescentic glomerulonephritis (NCGN). "Pauci-immune" in renal histology indicates the relative lack of immunoglobulin and complement deposition within the kidney by routine direct immunofluorescence (IF) and the lack of electron-dense deposits by electron microscopy (EM) [8]. Therefore, it has generally been assumed that the complement system is not involved in the pathogenesis of AAV, mainly because there is a paucity of immunoglobulin or complement deposition in vessel walls in AAV as compared to the substantial immunoglobulin and complement deposition that is observed in immune complex mediated glomerulonephritis and anti-glomerular basement membrane disease. However, recent observations in the anti-MPO induced vasculitis mouse model mentioned before suggest a critical role of complement activation in disease pathogenesis. In this model either anti-MPO IgG or splenocytes from MPO-deficient mice that are immunized with mouse MPO are passively transferred in syngeneic naive mice. This resulted in the development of pauci-immune crescentic glomerulonephritis and vasculitis [7]. Xiao et al. found that induction of glomerulonephritis with anti-MPO-IgG or anti-MPO splenocytes required activation of the alternative complement pathway. Complement depletion with cobra venom factor completely blocked the development of glomerulonephritis and vasculitis by injection of MPO IgG or transfer of anti-MPO splenocytes. Subsequently, the role of specific complement activation pathways was studied using mice deficient for the common pathway component C5, classical and lectin pathway component C4, and alternative pathway component factor B. These studies revealed that anti-MPO IgG induced NCGN is dependent on an intact alternative pathway. Whereas C4-deficient mice developed NCGN comparable to wild type mice, transgenic mice deficient for C5 or factor B were completely protected from disease induction. The authors also hypothesized that activation of neutrophils by ANCA-IgG causes the release of factors that activate complement [9]. Huugen et al. further demonstrated that inhibition of C5 activation attenuated disease development in the mouse model of anti-MPO-IgG-induced glomerulonephritis described before [10]. Schreiber et al. further found that recombinant C5a dosage-dependently primed neutrophils for ANCA-induced respiratory burst; C5a
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and the neutrophil C5a receptor may compose an amplification loop for ANCA-mediated neutrophil activation [11]. It remains inconclusive whether the complement system plays a role in the development of human AAV.

**Clinical aspects of AAV and pauci-immune crescentic glomerulonephritis**

ANCA-associated vasculitis is one of the multi-system autoimmune diseases in Caucasian populations. The annual incidence and point prevalence of renal vasculitis in Europe is 10-20/million/year and 150-200/million, respectively [12, 13]. Although individuals at all age can be affected by AAV, older people are more susceptible [14, 15]. Constitutional symptoms and signs of AAV include fever, fatigue, significant weight loss, myalgias and arthralgias. A variety of organs and systems can be involved in AAV, including respiratory tract, kidneys, gastrointestinal tract, nervous system, and ear-nose-throat (ENT). Kidneys and lungs are the two most vulnerable organs [16]. WG and MPA share many histological features, including NCGN that often leads to rapidly progressive glomerulonephritis. The classic triad of WG includes granulomatous inflammation of the respiratory tract systemic vasculitis, and NCGN. MPA has a similar spectrum of disease manifestations but there is an absence of granulomatous inflammation. RLV is characterized by the absence of extra-renal organ involvement. In CSS, three phases can be distinguished: allergic rhinitis and asthma, eosinophilic infiltrative disease, and systemic small vessel vasculitis.

As mentioned above, renal lesions in AAV are characterized by pauci-immune crescentic glomerulonephritis (CrGN). "Pauci-immune" in renal histology indicates the relative lack of immunoglobulin and complement deposition within the kidney by routine immunofluorescence (IF) and the lack of electron-dense deposits by electron microscopy (EM). Pauci-immune CrGN is the most common cause of rapidly progressive glomerulonephritis in adults and elderly patients [17]. Most cases of pauci-immune CrGN can be attributed to AAV [18]. However, a subgroup of patients with pauci-immune CrGN is persistently negative for ANCA. Studies on ANCA-negative pauci-immune CrGN have not provided sufficient data
due to the limited number of patients [19-25]. The pathogenesis of ANCA-negative pauci-immune CrGN has been rarely explored.

Survival of untreated AAV is dismal. For example, pulmonary involvement can lead to life-threatening pulmonary hemorrhage; renal involvement can lead to rapid loss of renal function. The cornerstone of treatment for ANCA vasculitis includes induction therapy with corticosteroids and intravenous (IV) or daily oral cyclophosphamide [26-29]. The majority of patients respond well to this therapy; remission is achieved in ~85% of patients [28, 30-32]. Unfortunately, many patients (up to 50%) experience a relapse within five years [33]. Even after patients progress to end-stage renal disease (ESRD), severe relapse of AAV, though not very common, can still occur [34]. Relapses, although often responding to therapy, contribute to mortality and end-organ damage of the patients [30]. Predictors of relapse include PR3-ANCA positivity, lung involvement, and upper respiratory tract involvement [30, 35].

ANCA are specific serological markers for AAV. As mentioned above, the combinations of a cANCA pattern by IIF with PR3-ANCA by ELISA and a pANCA pattern by IIF with MPO-ANCA by ELISA are very specific for AAV. PR3-ANCA are most frequent in patients with WG and MPO-ANCA in patients with MPA and RLV, although it is not always the case in different ethnicities [36]. However, the relationship between changes of ANCA level and relapse of AAV remains controversial. Many patients show decrease or disappearance of ANCA titers during periods of quiescence [37, 38]. A subsequent rise in ANCA titer or reappearance of ANCA has been suggested as being predictive of clinical relapse [39], suggesting that monitoring of changes in ANCA titer might be helpful in predicting relapses [40]. However, a recent multi-centre prospective cohort study with 156 patients with active Wegener’s granulomatosis showed that decreases in PR3-ANCA levels are not associated with shorter time to remission, and increases are not associated with relapse [41].

Biomarkers for assessing disease activity of patients with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) could be important for identifying relapse and guiding treatment. Although the exact pathogenesis of AAV has not been fully elucidated, it is well-known that neutrophils play an important role. In vitro studies have shown that ANCA could stimulate neutrophils to undergo a respiratory burst and
release free oxygen radicals and various proteases, which play a direct pathogenic role in vasculitic lesions [3-7]. In an anti-MPO antibody-induced mouse vasculitis model, it was suggested that neutrophils were necessary in the initiation of glomerulonephritis [42]. Therefore, it is reasonable to speculate that biomarkers of neutrophil degranulation might be associated with disease activity of AAV.

**Aim of the thesis**

This thesis consists of two parts. **Part I** focuses on the role of complement activation in the pathogenesis of ANCA-associated vasculitides. **Part II** focuses on clinical aspects of ANCA-associated vasculitides.

In **chapter 2**, we focus on advances in the understanding of the pathogenesis of ANCA-associated vasculitides.

Recent studies in animal models suggest that complement activation via the alternative pathway is one of the important contributing factors in the development of anti-MPO antibody-associated systemic vasculitis in mice [9, 10]. In **part I** of this thesis, the role of the complement system plays in human AAV is explored. In **chapter 3**, we discuss the updated evidence for activation of the complement system in ANCA-associated vasculitides and propose a working model that links ANCA, neutrophils and complement activation in causing an inflammatory amplification loop that may explain the severe leukocytoclastic inflammation that is typical for AAV. In **chapter 4**, we investigate C3c and C1q deposition by direct immunofluorescence in renal biopsy specimens from 112 patients with ANCA-associated pauci-immune glomerulonephritis. In **chapter 5**, we investigate the evidence for complement activation in renal biopsy specimens of patients with MPO-ANCA-associated pauci-immune glomerulonephritis by immunohistochemistry. It has been reported that the p38-mitogen-activated protein kinases (p38-MAPK) pathway controls the translocation of ANCA antigens to the cell surface during TNFα-mediated priming of neutrophils enabling subsequent ANCA-induced respiratory burst [11]. In **chapter 6**, we investigated the role of p38-MAPK pathway in C5a-mediated neutrophil activation in the pathogenesis of AAV.
Part II of this thesis investigates clinical aspects as well as biomarkers of ANCA-associated vasculitides. AAV is characterized by pauci-immune crescentic glomerulonephritis (CrGN). Most cases of pauci-immune CrGN can be attributed to AAV. However, a subgroup of patients with pauci-immune CrGN is persistently negative for ANCA. Chapter 7 reviews updated information on ANCA-negative pauci-immune CrGN, including prevalence, clinical manifestations, renal histopathology, outcome, and pathogenesis, in comparison with ANCA-positive pauci-immune CrGN. Chapter 8 and chapter 9 focus on clinical aspects of relapses of AAV. Relapses of AAV still are a challenge to physicians. Even after patients progress to end-stage renal disease (ESRD), severe relapse of AAV, though not very common, can still occur. In chapter 8, we describe 5 patients with ESRD who suffered from relapses of AAV, manifested as severe pulmonary hemorrhage, suggesting that disease activity and relapses of AAV should be monitored rigorously, even after patients progress to ESRD. Since relapses of AAV contribute to mortality and end-organ damage, early awareness and identification of relapses is crucial for improving prognosis. In chapter 9, we investigate the characteristics of organ involvement during relapses of AAV and find that relapses of AAV are likely to begin with the same organ as initial onset. It facilitates early recognition of relapses, both for physicians and patients. Chapter 10 and chapter 11 investigate biomarkers of disease activity of AAV. In chapter 10, we investigate whether neutrophil gelatinase associated lipocalin (NGAL), which is a 25kDa protein in secondary granules of neutrophils and a special biomarker of neutrophil degranulation [43-45], could be a useful biomarker for assessing disease activity of patients with AAV. In chapter 11, we investigate changes of avidity and titer of MPO-ANCA in sequential serum samples from some patients with AAV in an active phase of the disease, either at presentation or at the time of relapse, in comparison with remission.

In chapter 12, the data are summarized, and conclusions are drawn and discussed.
References