Pathogenetic and clinical aspects of ANCA-associated vasculitis
Chen, Min

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
CHAPTER 12

Summary
ANCA-associated vasculitides constitute a group of primary vasculitides, including Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS) and renal-limited vasculitis (RLV). These diseases are characterized by necrotizing small-vessel vasculitis in association with autoantibodies directed against neutrophil cytoplasmic constituents, in particular proteinase 3 (PR3) and myeloperoxidase (MPO). Kidneys and lungs are the two most commonly affected organs [1].

In this thesis, pathogenic and clinical aspects of ANCA-associated vasculitis have been studied. In part I of the thesis, the role of the complement system in AAV is explored. In part II, clinical characteristics and biomarkers for relapse of AAV have been investigated.

Below, the findings of this thesis are summarized.

In chapter 2, an overview of updated information on the pathogenesis of AAV is given. Clinical and in vitro and in vivo experimental data support a pathogenic role for ANCA in AAV. Additional clinical evidence for a pathogenic role of ANCA comes from the observation that patients with acute renal failure treated with plasma exchange had a lower risk for progression to end-stage renal disease (ESRD) than patients who receive intravenous methylprednisolone therapy [2, 3]. Recent data also suggest that antibodies to complementary PR3, probably cross-reacting with plasminogen, may induce PR3-ANCA [4]. Furthermore, a new ANCA, directed against human LAMP-2 has been described as a sensitive and specific marker for renal AAV [5]. In vivo, anti-LAMP-2 antibodies can induce pauci-immune necrotizing crescentic glomerulonephritis in rats. Rats developed both cross-reactive antibodies to LAMP-2 and crescentic glomerulonephritis when immunized with FimH, an adhesin from Gram-negative bacteria which has strong homology with human LAMP-2 [6, 7]. These recent studies have shown new light on the interaction of different ANCA and their pathogenic potential, but these findings should be confirmed and extended.
Pathogenic aspects of ANCA-associated vasculitis: role of complement

Recent observations, mainly in animal models, suggest a critical role of complement activation in the pathogenesis of ANCA-associated vasculitis [8-10]. In part I of this thesis, the role of the complement system in the pathogenesis of human AAV has been further explored.

In chapter 3, an overview of the involvement of the complement system in AAV is given. Evidence for complement activation in ANCA-associated vasculitis is discussed and a working model that links ANCA, neutrophils and complement activation in causing an inflammatory amplification loop that may explain the severe leukocytoclastic inflammation typical for ANCA-associated vasculitis is proposed.

In chapter 4, the clinical and pathological significance of complements deposition in renal histopathology of patients with ANCA-associated pauci-immune glomerulonephritis has been explored. By direct immunofluorescence microscopy, C3c could be detected in glomeruli in the specimens of nearly 1/3 of the patients. Compared with patients without C3c deposition, patients with C3c deposition had higher levels of urinary protein and poorer initial renal function. The proportion of patients with C1q deposition was relatively low, indicating that classical pathway activation is not the major pathway of complement system activation in human ANCA-associated glomerulonephritis. These findings suggest that complement deposition is not rare in renal histopathology of human ANCA-associated pauci-immune glomerulonephritis and is associated with more severe renal injury.

Since complement deposition was associated with more severe renal injury in human ANCA-associated pauci-immune glomerulonephritis, the next step should be to analyze the role of complement activation in the pathogenesis of human ANCA-associated pauci-immune glomerulonephritis. In chapter 5, complement activation in renal biopsy
specimens of patients with MPO-ANCA-associated pauci-immune vasculitis has been further investigated. Using immunohistochemistry, MAC, C3d, factor B and factor P could be detected in glomeruli and small blood vessels with active vasculitis of patients with pauci-immune AAV. The presence of MAC, the final product of complement activation, in glomeruli, arterioles and small arteries provided solid evidence for overall complement activation. Furthermore, by laser scanning confocal microscopy, co-localization of MAC with C3d was observed in glomeruli of patients with AAV, which demonstrates that complement activation is directly involved in the pathogenic process of renal vasculitis. MBL and C4d were not detected in patients with AAV. Combined with the lack of staining for C1q, our study did not support the involvement of both the classical pathway and the lectin pathway of complement activation. Furthermore, factor B and MAC, factor P and C3d co-localized well along the glomerular capillary wall and mesangial area in the glomeruli of patients with AAV. These findings suggest that the alternative pathway of the complement system is involved in renal damage of human pauci-immune AAV.

Huugen et al. further demonstrated that inhibition of C5 activation attenuated disease development in the mouse model of anti-MPO-IgG-induced glomerulonephritis [9]. Upon activation, C5 is spliced into two cleavage products. The C5b molecule is involved in the generation of C5b-9, the end product of the terminal complement pathway. The C5a molecule is a powerful chemoattractant for neutrophils, and has considerable neutrophil activating potential. Recent studies have suggested that C5a can dosage-dependently prime neutrophils for ANCA-induced respiratory burst; C5a and the neutrophil C5a receptor may compose an amplification loop for ANCA-mediated neutrophil activation [10]. In chapter 6, the signal transferring pathway of C5a-mediated neutrophil activation has been further investigated. C5a dose-dependently increased membrane expression of PR3 on neutrophils. Priming of neutrophils with C5a enhanced both PR3-ANCA and MPO-ANCA induced oxygen radical production. This effect is to
a large extent dependent on activation of the p38-MAPK pathway. Together with the observations in animals studies, these results indicate a prominent role for C5a-C5aR interactions in the pathogenesis of AAV.

Future studies include C5a receptor (C5aR) expression on glomerular endothelial cells and the effect of C5a on glomerular endothelial cells, including IL-6 and IL-8 secretion.

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

In the majority of patients, pauci-immune crescentic glomerulonephritis (CrGN) is a manifestation of ANCA-associated vasculitis. However, some patients with pauci-immune CrGN lack ANCA [11-14]. Chapter 7 reviewed updated information on ANCA-negative pauci-immune CrGN, including the prevalence, clinical manifestations, histopathology, and outcomes of ANCA-negative pauci-immune crescentic glomerulonephritis in comparison with that of ANCA-positive disease. It was found that approximately 10-30% of patients with pauci-immune CrGN lack ANCA. Patients with ANCA-negative pauci-immune CrGN have fewer extrarenal symptoms than patients who are ANCA-positive; ANCA-negative pauci-immune CrGN might be a distinct disease separate from ANCA-positive pauci-immune CrGN. Neutrophils are thought to have a major role in the pathogenesis of ANCA-negative pauci-immune CrGN.

The cornerstone of treatment for AAV has been the introduction of cyclophosphamide in combination with corticosteroids, which has improved the prognosis of AAV dramatically. However, many patients experience relapses despite long-term immunosuppressive therapy. Early identification of relapse and monitoring of disease activity, either from clinical observation or laboratory tests, is crucial for improving outcome of patients with AAV.

In this part, we first addressed the importance of monitoring relapses of AAV, even after patients progress to end-stage renal disease
It has been reported that patients with AAV who progress to ESRD are less likely to experience relapse of vasculitis than before the start of dialysis [15]. Therefore, when patients progress to ESRD, nephrologists may overlook the occurrence of relapses. In our clinical practice, we encountered 5 patients with ESRD who suffered from relapses of AAV, manifested as severe pulmonary hemorrhage. In chapter 8, these five patients with ESRD suffering severe pulmonary hemorrhage due to relapse of AAV are described. Nevertheless, we also observed that, after patients had progressed to ESRD, relapses including pulmonary hemorrhage were less likely to occur [16, 17]. Impairment of the immune response, especially cell-mediated response, in patients with ESRD might contribute to the decreased relapse rate of AAV [15]. Though not very common, severe pulmonary hemorrhage caused by relapses of AAV in patients with ESRD can occur and is life-threatening, suggesting that disease activity and relapses of AAV should be monitored rigorously, even after patients progress to ESRD.

In chapter 9, characteristics of organ involvement during relapses of AAV have been investigated. It was found that among 55 relapses in 38 patients with AAV, more than 70% of the relapses presented with involvement of the same organ as at the initial onset. Although the mechanisms are not clear, it was speculated that the pathogenic pathway leading to relapses and initial manifestation of the disease might be the same. Our current therapy might just suppress the disease rather than clearing the etiologic trigger of the disease. Localized subclinical disease might persist during an apparent remission and progress to fulminant active disease after remission. These observations might provide physicians with a useful tool to recognize relapse of AAV. Also patients can be also educated to pay special attention to the symptoms that occurred at the initial onset of the disease for early awareness of relapse.
Biomarkers for assessing disease activity of patients with AAV are important in identifying relapse and guiding treatment. ANCA-induced neutrophil activation and degranulation play an important role in the pathogenesis of AAV. Circulating neutrophil gelatinase associated lipocalin (NGAL) is a biomarker of neutrophil degranulation. In chapter 10, we investigated whether NGAL is a useful biomarker for assessing disease activity of patients with AAV. The levels of serum NGAL in patients at initial onset and relapse were both significantly higher than that at remission. Levels of serum NGAL closely correlated with Birmingham Vasculitis Activity Score (BVAS) as well as with levels of ESR, CRP and ANCA. Moreover, serum NGAL levels more closely correlated with BVAS than ESR, CRP and ANCA. These findings suggest that circulating NGAL can be considered a useful biomarker for assessing disease activity of patients with AAV. A large prospective study is required to confirm these findings.

In chapter 11, we investigated changes of avidity and titer of MPO-ANCA in sequential serum samples from some patients with AAV at initial onset and relapse as well as during remission. The titer of MPO-ANCA was not significantly different between initial onset and remission. The avidity of MPO-ANCA in the active phase was not significantly different from that during remission. No significant correlation could be found between avidity and level of BVAS, times of relapse, number of organ involvement, serum creatinine, or CRP. These findings suggest that avidity and titre of MPO-ANCA do not decrease significantly during remission in AAV, possibly indicating that the underlying antigenic stimulation persists, which might be a reason for recurrent relapses. Further studies should also focus on patients with PR3-ANCA.

Final conclusion

Activation of the complement system via the alternative pathway has been involved in the development of human MPO-ANCA-associated vasculitis. C5a, which plays a pivotal role in the pathogenesis of AAV,
mediates neutrophil activation through the p38-MAPK pathway. Severe relapse of AAV can occur even after patients progress to ESRD. Circulating NGAL could be used as a useful biomarker for assessing disease activity of patients with AAV. Avidity and titer of MPO-ANCA do not decrease significantly during remission in AAV, indicating that the antigenic stimulant persists, which might be the reason for recurrent relapses.

References

17. Chen M, Yu F, Zhao MH. Relapses in patients with antineutrophil cytoplasmic autoantibody-associated vasculitis: likely to begin with the same organ as initial onset. J Rheumatol 2008; 35: 448-50