ANCA-associated glomerulonephritis : insights into etiology, pathogenesis, and prognosis

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Chapter 1

General introduction

This thesis focuses on different aspects of renal involvement in anti-neutrophil cytoplasm autoantibodies (ANCA)-associated vasculitis, in particular histological lesions and clinical outcome. This chapter introduces the reader to some of the many aspects of this disease and aims to put ANCA-associated vasculitis and its various clinical and histopathological presentations into broader perspective. The paragraphs of this chapter further consider current hypotheses on pathogenesis and up-to-date views on treatment and prognosis. Considerations on etiology will be discussed in chapter 6.

Vasculitis

Classification of systemic vasculitis

Vasculitis refers to an inflammatory process in the blood vessel wall. It is a spectrum of different diseases often associated with destruction of the vessel wall and occlusion of the vascular lumen. Since any vessel in any organ can be affected, clinical manifestations can vary widely. Therefore, vasculitis is hard to diagnose, and in clinical practice, is often not recognized at first. Furthermore, within the spectrum of vasculitides, it is often not clear-cut which disease the patient is suffering from. Nevertheless, this distinction is important to establish, since the different vasculitides are treated differently and vary in prognoses [1]. There is a lack of knowledge on the etiology of most (if not all) vasculitides. Therefore, classification can not be based on the cause of the disease, but only on the similar occurrence of symptoms and signs in groups of patients. Over the last fifteen years, progress has been made in accurately classifying the different diseases, mainly by international consensus on nomenclature and serology [2], but up to this day diagnosing individual patients remains hard.

In 1994, during the Chapel Hill consensus conference on the nomenclature of systemic vasculitis, an attempt was made to unify nomenclature of the different forms of vasculitis [2]. A classification system was made, based on the size of vessels affected by the different diseases. Distinction was made between large-vessel vasculitis, affecting the aorta and the largest arterial branches directed toward major body regions, medium-sized-vessel vasculitis, affecting the main visceral arteries and their branches, and small-vessel vasculitis, affecting small arteries, arterioles, capillaries, and venules. Of note is that all three categories of vasculitis can affect arteries. The utility of this classification for diagnosing the individual patient can be questioned. In fact, the authors claim the definitions are not classification criteria, but a guideline that can be used as a basis for classification systems in different studies [2]. However, the

different ANCA-associated small-vessel vasculitides might still be one disease with each patient having an individual clinical and histological profile of the disease. Moreover, these definitions are not diagnostic criteria, leaving it a difficult task to diagnose individual patients.
This thesis will only focus on small-vessel vasculitis and only on the disease-subcategories that are ANCA-related: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and renal-limited vasculitis (RLV). The Churg-Strauss syndrome (CSS) is not studied in this thesis, since histological features and clinical manifestations are different from the other ANCA-associated small-vessel vasculitides.

Clinical picture
ANCA-associated vasculitis is, with an incidence of 15-30 patients/million per year, the most frequently occurring primary small-vessel vasculitis. It is usually diagnosed between 50 and 60 years of age, but can occur at any age. Early in the course of their disease, many vasculitis patients describe a 'flu-like' syndrome. General signs and symptoms include fever, malaise, myalgias, and migratory arthralgias. Small-vessel vasculitis can involve venules, capillaries, and arterioles, but also small arteries and veins anywhere in the body [2]. The respiratory tract, kidneys, skin, gut, skeletal muscles, and peripheral nerves, for instance, often participate in the disease process of small-vessel vasculitis. Clinically, this disease entity includes the diagnoses of WG, MPA, and CSS. WG and MPA share many histological features, such as necrotizing glomerulonephritis which often leads to progressive renal failure. The classic triad of systemic small-vessel vasculitis, granulomatous inflammation of the respiratory tract, and necrotizing glomerulonephritis is highly suggestive of a diagnosis of WG [3]. It can be distinguished from other vasculitides by the presence of necrotizing granulomatous inflammation in the absence of asthma. MPA has a similar spectrum of disease manifestations, but there is an absence of granulomatous inflammation [2]. However, in WG granulomatous inflammation can often not be detected. Differentiation between these two diagnoses before starting treatment is not clinically relevant, since treatment is essentially the same. These arguments strengthen the hypothesis that WG and MPA should be regarded as two forms of a spectrum of ANCA-associated diseases, instead of two separate entities. If an ANCA-positive patient has pulmonary infiltrates with pauci-immune crescentic necrotizing glomerulonephritis on renal biopsy, treatment should not be delayed [1]. RLV is

also distinguished and is characterized by the absence of extra-renal organ involvement. In CSS, three phases can be distinguished: allergic rhinitis and asthma, eosinophilic infiltrative disease resulting e.g. in gastroenteritis or eosinophilic pneumonia, and systemic small-vessel vasculitis with granulomatous inflammation [4-6]. Neuropathy and cardiac disease are more frequent than in WG and MPA, while renal disease is less frequent [4;6]. CSS will not be further discussed in this thesis.

Discovery of small-vessel vasculitis

Vasculitis as it is known today was discovered through two historical pathways of investigation that eventually crossed each other's way. One sprouted from the description of necrotizing arteritis [7], while the other found its roots in the description of purpura [8-11]. Eventually, they were both considered to be part of the spectrum of systemic vasculitides.

In 1866, Kussmaul and Maier reported a patient with necrotizing arteritis with nodular inflammatory lesions that affected medium-sized and small arteries in the body, a condition which they called periarteritis nodosa (later known as polyarteritis nodosa or PAN) [12]. The first case of Wegener's granulomatosis was described by Klinger in 1931 [13], and later in more detail by Wegener in 1939, describing granulomatous disease [14]. A related disease which could be distinguished on the basis of predominantly asthma and eosinophilia was first described in 1951 by Churg and Strauss after whom the syndrome was named [7].

In 1948, Davson et al. described a microscopic form of PAN with renal involvement, characterized by segmental crescentic glomerulonephritis [15]. Later, in 1954, Godman and Churg distinguished Wegener's granulomatosis, the 'microscopic form of periarteritis' (renamed microscopic polyangiitis in 1994 [2]), and polyarteritis nodosa [3]. By 1980, the term small-vessel vasculitis was well established.

Already in 1808, the first description of a symptom of small-vessel vasculitis was the distinction between infectious and non-infectious purpura, with a predilection for the lower extremities [8]. Henoch [9;10] and Schönlein [11], after whom the disease was later named, amongst others, reported on the spectrum of signs and symptoms associated with purpura. In the early 1900s, Osler was the first to discover that these symptoms were caused by necrotizing inflammation of the wall of small vessels [16;17], designating this disease to the small-vessel vasculitides.

Immune-complex and pauci-immune small-vessel vasculitis

With the development of immunofluorescence microscopy, the identification of pathogenic antibodies in tissue and serum became part of standardized clinical practice. Examples are the discovery of antibodies directed against the glomerular basement membrane in Goodpasture's syndrome [18-21], deposition of immune complexes in cryoglobulinemic vasculitis [21], and IgA deposits in Henoch-Schönlein purpura [22]. These findings are supported by the concept that in the pathogenesis of some forms of small-vessel vasculitis immune-complex formation is involved. However, in a number of small-vessel vasculitides, in immunofluorescence microscopy, the presence of immune complexes is scarce, with a weak granular staining pattern of IgG, IgM, or complement [23;24], and often even absent. This is seen in WG, MPA, and Churg-Strauss syndrome. The condition is designated 'pauci-immune' [24-26]. However, animal studies have demonstrated that immune complexes are present at the site of injury in an early stage of the disease [27]. Whether immune complexes have never been present at the site of vessel injury or whether they have been resolved by the time patients develop clinical manifestations of ANCA-associated vasculitis is still subject of debate [28].

ANCA and its antigens

C-ANCA and P-ANCA

A major breakthrough in the field of small-vessel vasculitis came in 1985, when WG was associated with antibodies directed against lysosomal constituents of neutrophils and monocytes: the ANCA [29]. A technique was developed by which two distinctive patterns can be visualized by using indirect immunofluorescence (IIF), incubating ethanol-fixed neutrophils from healthy donors with patients' sera [30-32]. One pattern is characterized by diffuse fine granular staining of the cytoplasm with an accentuation of staining in the central area of the cell between the nuclear lobes, which was named the cytoplasmic staining pattern (C-ANCA). This phenomenon was first described to be specific for WG by van der Woude et al. [29]. The perinuclear staining pattern (P-ANCA) is characterized by staining of the nucleus, the perinuclear area, or both. The P-ANCA staining pattern is an artifact caused by shifting of cationic cytoplasmic proteins towards the negatively charged nuclear membrane, upon ethanol fixation [33]. This pattern was later described by Falk and Jennette [34]. The two staining patterns are visualized in Figure 1. Any positive staining not showing a clear C-ANCA or P-ANCA pattern is referred to as an atypical staining pattern.

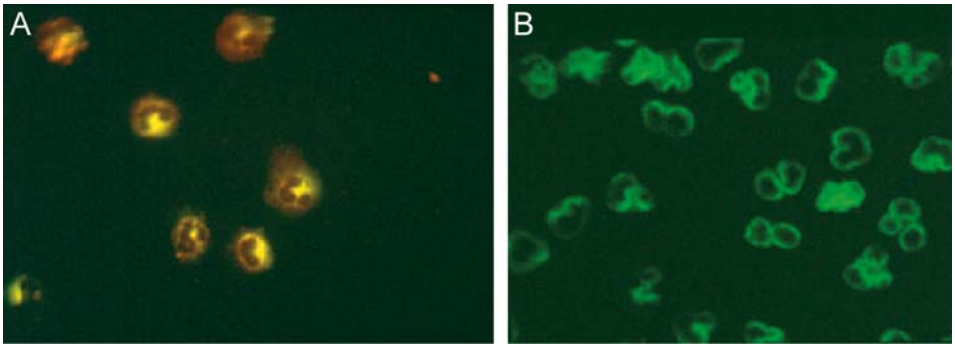

Figure 1. Cytoplasmic components of ethanol-fixed neutrophils, stained by indirect immunofluorescence. A. A characteristic granular pattern of fluorescence is seen: C-ANCA. B. This fluorescence pattern shows perinuclear staining: P-ANCA. Reprinted by permission from Macmillian Publishers Ltd: Nat Clin Pract Rheumatol 2(12): 661-70 © (2006) Kallenberg CG, Heeringa P, Stegeman C. Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides.

PR3-ANCA and MPO-ANCA

As determined by enzyme-linked immunosorbent assay (ELISA), ANCA can be directed against serine proteases such as proteinase-3 (PR3-ANCA), but also against a number of other antigens such as myeloperoxidase (MPO-ANCA), human leukocyte elastase (HLE-ANCA) [35], lactoferrin [36], cationic antimicrobial protein (CAP57) [37], cathepsin G [37] and bactericidal/permeability increasing protein (BPI-ANCA) [38].

ANCA detection and clinical disease

MPA is characterized by a non-granulomatous systemic vasculitis [39], and is often associated with ANCA directed against myeloperoxidase (MPO-ANCA) [34;39]. WG is characterized by a granulomatous systemic vasculitis with upper airway involvement [2;40]. ANCA are directed against proteinase-3 (PR3-ANCA) in most WG cases [41]. The ELISA has added substantially to diagnosing ANCA-associated vasculitis, with a diagnostic sensitivity higher than IIF [42].

The link between ANCA pattern by ELISA or IIF and clinical diagnosis is not always clear-cut. Although classical WG is characterized by PR3-ANCA involvement and a C-ANCA pattern, this relationship is not always obvious (Table 1). Classic MPA is characterized by MPO-ANCA and a P-ANCA pattern. A P-ANCA staining pattern can also occur in many non-vasculitic diseases, for instance systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, HIV infection, and chronic liver disease. The occurrence

Table 1. Overview of different diagnoses and the occurrence of different ANCA-patterns as determined by IIF and ELISA. This table was adapted from ref 39.

Diagnosis	C-ANCA	P-ANCA	PR3-ANCA	MPO-ANCA
Wegener's granulomatosis	64%	21%	66%	24%
Microscopic polyangiitis	23%	58%	26%	58%
Renal-limited vasculitis	36%	45%	50%	64%

of the different ANCA patterns in different vasculitides was described by Hagen et al. [39] and is listed in Table 1.

Pathogenesis

Are ANCA pathogenic?

The question whether ANCA are pathogenic has been a subject of discussion since their discovery in 1985 [29]. Most ANCA researchers believe they are important in the pathogenesis of ANCA-associated vasculitis. There is some clinical evidence for this presumption, although this is rather suggestive than definitive. First of all, approximately 90% of patients with WG, MPA and renal limited vasculitis have circulating ANCA [39]. Secondly, ANCA can be induced by certain drugs, such as propylthiouracil, with a concurrent onset of systemic small-vessel vasculitis. Upon cessation of these drugs, ANCA titers and clinical disease resolve (chapter 6). In addition, two recent reports from the European Vasculitis Study Group (EUVAS) have provided evidence that plasma exchange is beneficial as adjunctive treatment [43]. Even in severe clinical and histologic renal disease, the likelihood of renal recovery was significantly improved with plasma exchange (chapter 3). However, what is responsible for ANCA production in the first place is still unknown. The most important hypotheses on the etiology of ANCA-associated vasculitis are described in chapter 6.

In vitro evidence for a pathogenetic role of ANCA in vasculitis is based on the observation of many laboratories that ANCA IgG can cause activation of MPO- and PR3-containing neutrophils and monocytes. These cells then become activated and release or express molecules that are mediators of inflammation [44-48]. This process is visualized in Figure 2.

In vivo evidence for pathogenecity of ANCA started with the introduction of the first successful animal model for ANCA-associated vasculitis in 1993 [27]. However in this study, MPO-ANCA did not cause disease in Brown-Norway rats immunized with human MPO. The induction of a neutrophil extract containing MPO and hydrogen peroxide did induce severe necrotizing crescentic glomerulonephritis in MPO-immunized rats [27]. A major breakthrough

in unravelling the mystery of MPO-ANCA being pathogenic came in 2002 [49]. An anti-murine MPO immune response was generated in MPO-/- mice by immunizing them with murine MPO. Adoptive transfer of anti-MPO-positive splenocytes into Rag2-/- mice, that lack functioning T- and B-cells, led to circulating MPO-ANCA and the development of crescentic glomerulonephritis

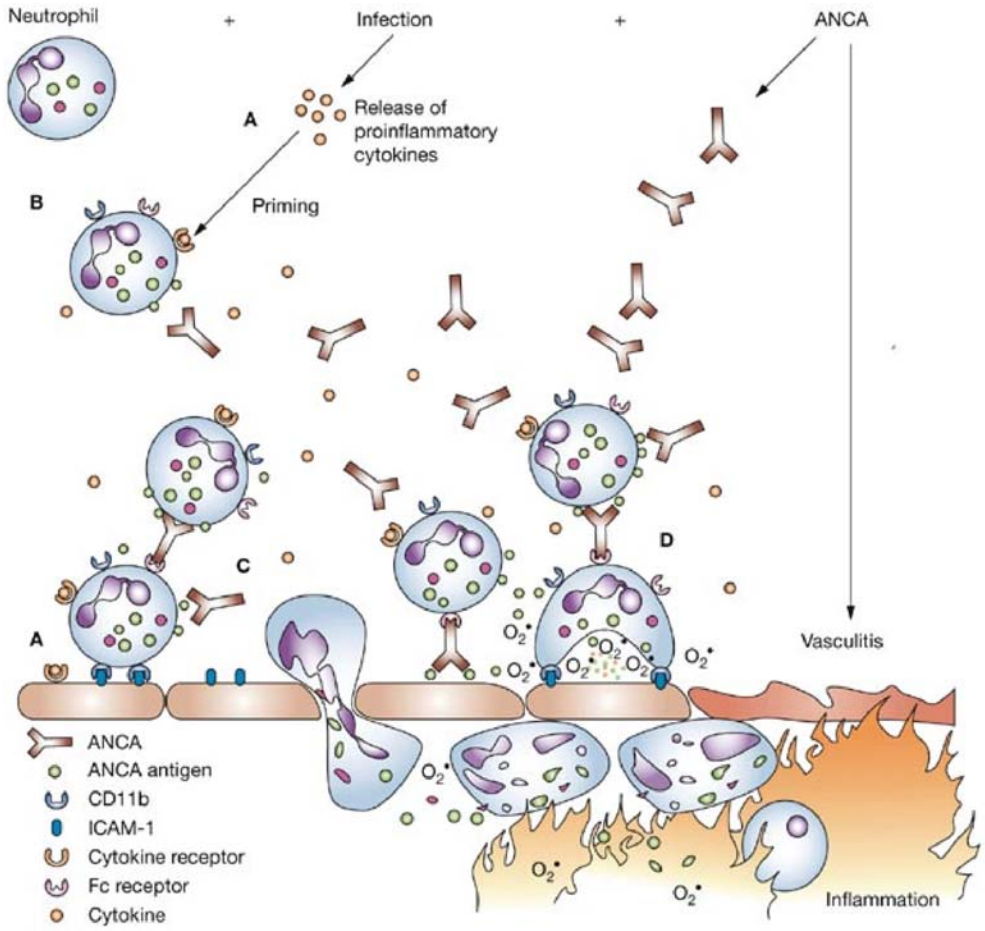

Figure 2. Schematic representation of the neutrophil responses that are putatively involved in the pathogenesis of ANCA-associated small-vessel vasculitis.
Reprinted from Trends Immunol 26: 561–564 © (2005) Heeringa P, Huugen D, Tervaert JW. Anti-neutrophil cytoplasmic autoantibodies and leukocyte–endothelial interactions: a sticky connection?, by permission from Elsevier Ltd. and reprinted by permission from Macmillian Publishers Ltd: Nat Clin Pract Rheumatol 2(12): 661-70 © (2006) Kallenberg CG, Heeringa P, Stegeman C. Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides.

in these mice. In a separate experiment, Rag2-/- and wild-type mice were injected with purified anti-MPO IgG inducing pauci-immune focal necrotizing crescentic glomerulonephritis, more outspoken in the Rag2-/- mice [49]. These experiments led to the conclusion that ANCA can induce vasculitis, even in the absence of T- and B-cells [50;51]. Furthermore, human MPO immunized WKY rats developed anti-human MPO antibodies cross-reacting with rat MPO and inducing pauci-immune necrotizing crescentic glomerulonephritis over time [52]. Attempts at creating an animal model for vasculitic disease associated with PR3-ANCA have not yet been successful. Recently, it was demonstrated that mice and rats immunised with chimeric human/mouse PR3 produced autoantibodies to mouse PR3 [53]. However, this did not result in any gross pathology in the kidneys nor the lungs of these animals. A possible explanation as to why these experiments have failed might be that murine PR3 is more similar to murine and human neutrophil elastase than to human PR3. This might result in an absence of expression of murine PR3 at the neutrophil membrane of the mouse under inflammatory conditions [54]. Another explanation might be that there are differences in ionic strength between murine MPO, human PR3, and murine PR3, which could explain differences in tissue retention and could account for different pathophysiological effects [55;56].

The pathogenesis of fibrinoid necrosis

One of the histologic hallmarks in renal biopsies in ANCA-associated vasculitis is a lesion named fibrinoid necrosis. It is assumed to be a lesion containing necrotic material with a fibrin-like structure, however the exact components of the lesion have not been identified yet [57]. This lesion can not only be found in glomeruli, but also in vessel walls. The pathogenic pathway of fibrinoid necrosis is still a subject of discussion. Nowadays, the most common pathogenic theory (as visualized in Figure 2) is that proinflammatory cytokines and chemokines prime neutrophils and monocytes, causing upregulation of neutrophil adhesion molecules (e.g. CD11b) and translocation of the ANCA antigens from the lysosomal compartments to the cell surface [50]. Proinflammatory cytokines also upregulate endothelial cell adhesion molecules, such as selectins, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) [58]. Dimer formation between neutrophils takes place by the interaction of the F(ab')2 portion of ANCA with ANCA antigens on the endothelial cell surface and of the Fc part of the antibody with the Fc receptors on the neutrophil. These interactions activate neutrophils,

promoting the adherence to and transmigration through the vessel wall. Furthermore, ANCA-mediated neutrophil activation causes these cells to undergo a respiratory burst with the release of reactive oxygen species as well as degranulation of azurophilic granules, and the release of pro-inflammatory cytokines [59-62]. This release of proteolytic enzymes, including the ANCA antigens PR3 and MPO, leads to endothelial cell damage and vasculitis.
A recent hypothesis postulated that PR3 and MPO not only bind to endothelial cells, but are also internalized in these cells [50]. Internalization of PR3 causes endothelial cell apoptosis, whereas internalization of MPO causes generation of oxygen radicals [50;63]. The encounter of ANCA with its antigens, located on the surface of endothelial cells of tissue matrix, results in the formation of focal immune complexes. These immune complexes recruit neutrophils which dissolve the immune complexes. The presence of the transient immune complexes together with the activation of the alternative pathway of the complement system following ANCA-neutrophil interaction probably initiate an inflammatory amplification loop, enhancing the recruitment of more neutrophils, eventually leading to necrotizing vasculitis [50;46].
Another hypothesis that parallels the former, postulated that after the endothelial cells are damaged and vasculitis is initiated, more mononuclear leucocytes are assembled which augments the vascular inflammation and hereby the damage. After this event, the neutrophils (in self-limited inflammation) normally go into apoptosis and are cleared by macrophages [65-68]. This, however, is interfered with by the activation of ANCA accelerating apoptosis and secondary necrosis in cytokine-primed neutrophils [60;69-70], inhibiting clearance of apoptotic cells by macrophages [69]. This finally results in the formation of fibrinoid necrosis.

The involvement of T-cells
Increasing evidence accumulates that next to neutrophils, endothelial cells, and macrophages, T-cells play an important role in the pathogenesis of ANCA-associated vasculitis [46;71]. Controversially, experiments in animal models have demonstrated the induction of vasculitis by MPO-ANCA in the absence of T-cells. This demonstrates that these cells are not needed for the induction of vasculitis (in animals), but this does not rule out a role in a more downstream process in the pathogenesis of the disease. However, there is evidence for a role for T-cells in early stages of vasculitic disease, but much of their function is still undiscovered.

The help of T-cells is required in the ANCA production [72]. The predominance of the subclasses IgG1, IgG3, and IgG4 suggests an antigen-driven T-cell dependent immune response [72;73]. T-cells were also found to be oligoclonically expanded in systemic necrotizing vasculitis [74]. In WG, serum levels of sIL-2R, sCD4, sCD8, and sCD30 are elevated, indicating T-cell activation, and are correlated with disease activity [75-78]. However, even in remission and despite treatment, activation markers on T-cells are upregulated [79;80]. In the presence of PR3 and MPO, T-cells proliferate [81;82]. They also infiltrate vasculitic and granulomatous lesions [83-86], and in the kidney T-cell accumulation is correlated with renal impairment [85]. Treatment with monoclonal antibodies directed against T-cell markers is effective in inducing remission [87-90].

The theory of autoantigen complementarity

A recent, remarkable finding was that patients with ANCA directed against PR3 not only harbor antibodies to PR3, but also to the peptide translated from the antisense DNA strand of PR3 (complementary PR3, cPR3), as visualized in Figure 3. Complementary portions of the PR3 encoding gene PRTN3 were found in a number of microbial and fungal organisms, including *Staphylococcus aureus* and Ross River virus [91]. The role of *S. aureus* as an etiological factor in ANCA-associated vasculitis is discussed in chapter 6. The theory of autoantigen complementarity encompasses autoimmunity as a consequence of an immune response to a protein whose amino acid sequence is complementary to that of a self protein [91]. In vasculitis patients, the idiotypic antibody directed against cPR3 evokes a second immune response with newly-formed antibodies directed against the active binding of the idiotypic antibodies. The binding site of these newly-formed antibodies (PR3-ANCA) is complimentary to PR3 and these antibodies can react with PR3. This theory was also tested *in vivo*. Mice immunized with cPR3 developed anti-cPR3 and anti-PR3 antibodies [91]. Whether therapies directed against the anti-idiotypic antibody are helpful remains to be seen. The observation that intravenous immunoglobulins (IVIg) inhibit ANCA-induced neutrophil activation [92;93] and that IVIg reduces disease activity in persistent ANCA-associated vasculitis may be based on this concept [94]. The theory of autoantigen complementarity provides new clues to the thought of the concept of autoimmunity, including its etiology.

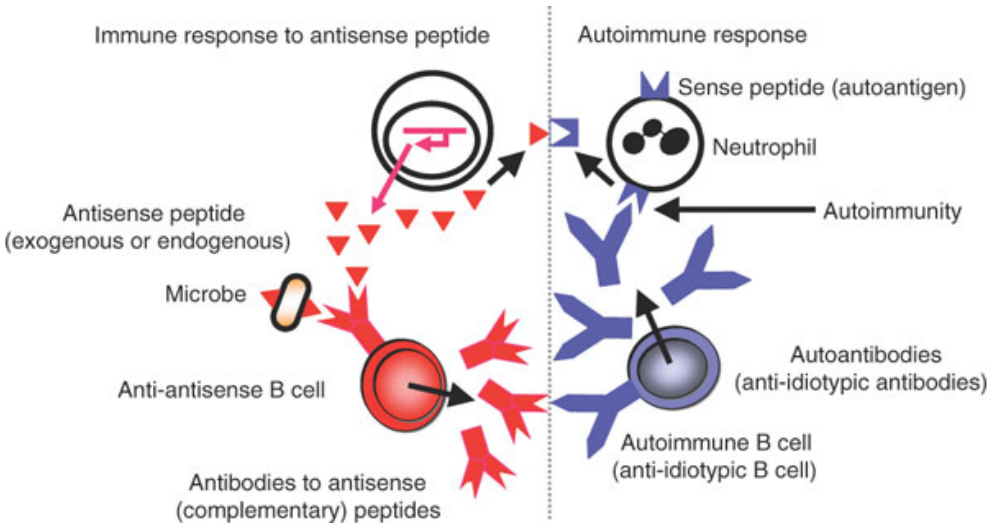

Figure 3. The theory of autoantigen complementarity, a new mechanism for the development of autoimmunity whereby proteins complementary to autoantigens are initiators of disease. Autoimmunity is a consequence of an immune response to a protein whose amino acid sequence is complementary to that of a self-protein. The immunogen, which elicits the initial immune response (idiotypic response), is complementary in amino acid sequence to the autoantigen. This idiotypic antibody elicits a second immune response (anti-idiotypic response) in which anti-idiotypic antibodies or autoanti-idiotypes are produced. The anti-idiotypic antibodies are now autoantibodies that react with self-antigen, resulting in autoimmunity. Reprinted with permission from Macmillian Publishers Ltd: Nat Med 10: 72-79 © (2004) Pendergraft WF, III, Preston GA, Shah RR, Tropsha A, Carter CW, Jr., Jennette JC, Falk RJ. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3.

Histopathology of renal lesions

In biopsies of kidneys affected by ANCA-associated vasculitis, a variety of glomerular and tubulo-interstitial lesions can be found. Although the glomerular lesions have been studied extensively, the involvement of the tubules and the interstitium in the disease process may be of importance too, especially with regard to predicting renal outcome. This is discussed in chapter 2, 3, and 4.

A hallmark in renal histopathology in patients suffering from ANCA-associated vasculitis is fibrinoid necrosis. This lesion is considered to be caused by vasculitis of the capillary tuft [95] and can be revealed for instance as red spots in fibrin Lendrum staining (Figure 4A). Because of its fibrin-like structure upon microscopical examination [96], this lesion was called fibrinoid, however it is not composed of fibrin or fibrinogen alone, but also of extracellular matrix molecules [57]. It has been suggested that this lesion is the result of a disturbed

interaction of endothelium with ANCA-antigens, expressed by TNF-α primed neutrophils [97]. Damage of the vessel wall leads to an intracapillary thrombotic process acculumating clotting factors and eventually fibrin, resulting in disintegration of the mesangium [98] and rupture of the capillary tuft [99]. Others claim that glomerular thrombosis plays a primary pathogenetic role in the development of ANCA-associated glomerulonephritis [26]. A hypothesis we propose is that fibrinoid necrosis is part of a healing process, founded by the discovery that fibrinoid necrotic lesions of C-ANCA patients contain the ED-B-positive fibronectin isoform [100]. This isoform is involved in the process of angiogenesis and plays a role in tissue repair mechanisms and scar formation similar to what has been described for wound healing [101;102]. Also the upregulation of integrin α6β1, and the downregulation of α5β1 in fibrinoid necrotic lesions of ANCA-associated vasculitis patients could well reflect tissue repair reactions [103]. This hypothesis is further supported by the fact that fibrinoid necrosis bears regenerative capability [104] and its presence is a predictor of better renal outcome in patients presenting with mild to moderate renal involvement [105]. Another histopathological hallmark is extracapillary proliferation, a lesion seen in a number of glomerular diseases, such as anti-GBM glomerulonephritis, lupus nephritis, and IgA nephropathy. Extracapillary proliferation refers to cells of epithelial and inflammatory origin proliferating into Bowman's space (Figure 4B). This process which is also referred to as crescent formation, finds its origin in fibrin leakage from the glomerular tuft through the damaged glomerular basement membrane [98]. Consequently, fibroblasts migrating from the interstitium through defects in the glomerular capsule are held responsible for the formation of scar tissue following fibrotic changes [106]. The deposition of fibrin attracts macrophages by chemotaxis [107], exerting pro-coagulant activity. In its turn, this leads to intraglomerular fibrin deposition [108], completing the vicious circle of extracapillary proliferation and compressing the glomerular tuft, resulting in a decrease of filtration surface. In parallel with this process, predominantly cellular crescents (Figure 4C) that may still recover to normal glomeruli, surpass a point at which these become irreversibly damaged. Fibroblasts enter Bowman's space with the evolvement of a fibrocellular crescent [107;109;110]. Consequent collagen accumulation will lead to the formation of fibrous crescents (Figure 4D) and eventually glomerulosclerosis (Figure 4E). Crescents can be distinguished by those that cover less than 50% of the glomerular surface in one cross-section, referred to as segmental crescents (Figure 4C), and those that cover more than 50% of the surface, referred to as

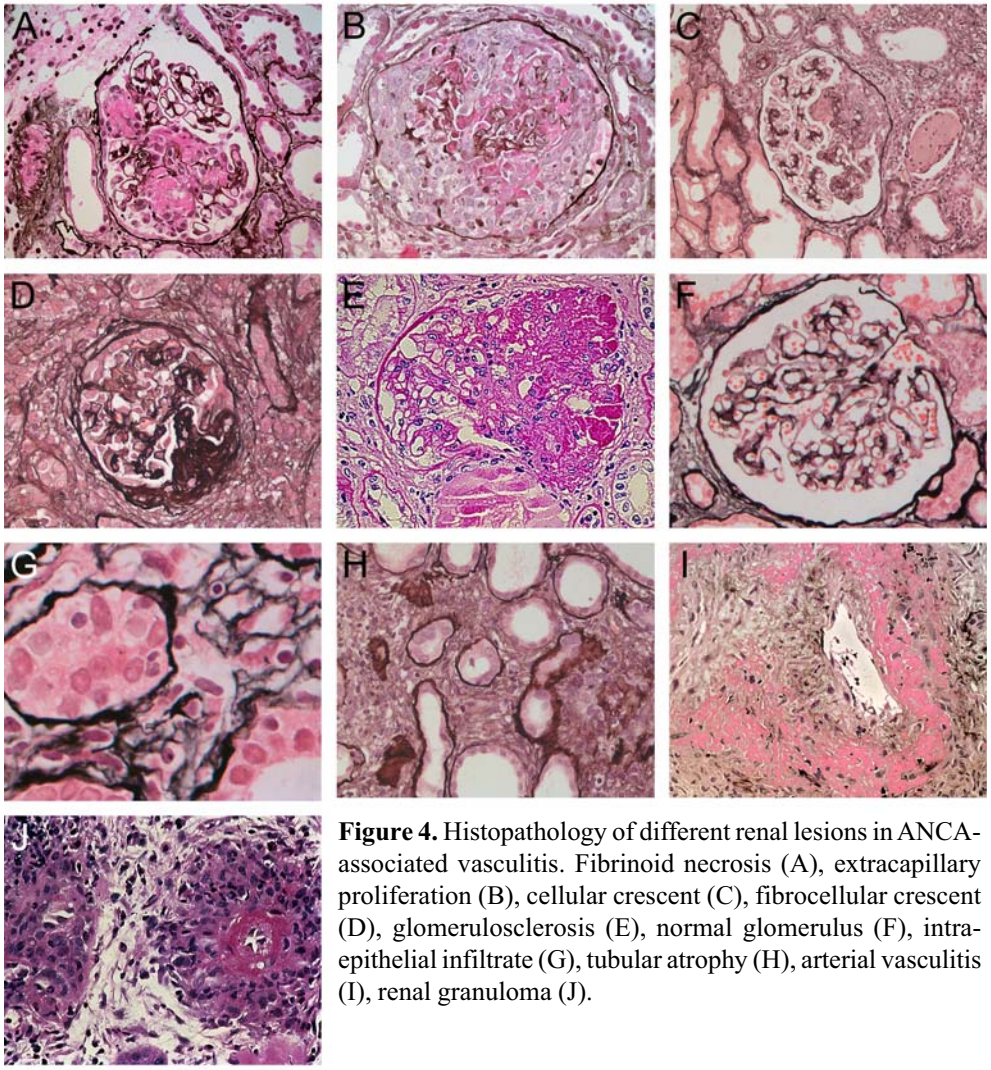

Figure 4. Histopathology of different renal lesions in ANCA-associated vasculitis. Fibrinoid necrosis (A), extracapillary proliferation (B), cellular crescent (C), fibrocellular crescent (D), glomerulosclerosis (E), normal glomerulus (F), intra-epithelial infiltrate (G), tubular atrophy (H), arterial vasculitis (I), renal granuloma (J).

circumferential crescents (Figure 4B). Due to the focal nature of the disease, also normal glomeruli can be found in the biopsy (Figure 4F).
The combination of fibrinoid necrosis, extracapillary proliferation and the absence of immune deposits in indirect immunofluorescence (IIF) techniques, is known as pauci-immune crescentic necrotizing glomerulonephritis, the histological diagnosis of kidney disease in ANCA-associated vasculitis.
The damage in the tubulointerstitium has often been considered as secondary in histopathological studies on ANCA-associated vasculitis, however these turn out to be very important when it comes to predicting renal outcome over

time in patients presenting with severe renal involvement (chapter 2, 3, and 4). With regard to tubulointerstitial lesions a distinction can be made between acute and chronic lesions. Intraepithelial infiltrates -often referred to as tubulitis- (Figure 4G), tubular necrosis, interstitial edema, and interstitial infiltrates are associated with active disease. Tubular atrophy (Figure 4H) and interstitial fibrosis are associated with chronic damage.
Vasculitis of arteries and arterioles is detected in approximately 25% of renal biopsies from patients with ANCA-associated vasculitis (Figure 4I). This low number can be explained by the small size of the biopsy and the focal nature of the disease, and consequently, the relatively low chance of an affected small vessel in the biopsy specimen being present. Alternatively, renal blood vessels may be less prone to vasculitis, since not all vessels in the body are equally involved.
In approximately 5% of renal biopsies from patients suffering from Wegener's granulomatosis, renal granulomas are observed [111] (Figure 4J). However, these can also be found in other vasculitides and a number of other inflammatory diseases, such as sarcoidosis, tuberculosis, hypersensitivity, and around foreign bodies [112], and should therefore not be regarded as pathognomonic of the disease.

Treatment and prognosis
Untreated, generalised ANCA-associated vasculitis follows a progressive course to vital organ failure with a fatal outcome [113]. In the early 1970's, oral administration of the toxic cyclophosphamide in combination with corticosteroids was already appreciated as an effective treatment for ANCA-associated vasculitis [114]. Seventy-five percent of patients achieved complete remission, but a major point of concern was that 50% of these relapsed within 5 years [115]. Another point of concern was the adverse effects associated with cyclophosphamide and steroid treatment, especially cyclophophamide toxicity. Hemorrhagic cystitis, bladder cancer, and opportunistic infection during leukopenia are clinical manifestations of these adverse effects [58;115-117]. Other adverse effects include nausea, vomiting, neutropenia, alopecia, male and female infertility, leukaemia, and lymphoma. In the CYCAZAREM (cyclophosphamide versus azathioprine for maintenance of remission) trial ran by the European Vasculitis Study Group (EUVAS), cyclophosphamide was shown to be safely replaced by azathioprine after induction of remission in patients with mild to moderate renal disease (200 μmol/L ≤ serum creatinine < 500 μmol/L). A similar number of patients relapsed within 18 months after

diagnosis, but less toxicity was observed in the patients who switched to azathioprine treatment during remission (10% vs. 18%) [118]. To reduce cyclophosphamide toxicity, different ways of administration of cyclophosphamide for the induction of remission were studied by the EUVAS [119]. Although in patients treated with daily oral cyclophosphamide (2 mg/kg/day) the cumulative dose was twice as high as in those treated with intravenous pulse cyclophophamide (15 mg/kg every 2 to 3 weeks), no differences were observed between cumulative survival, time to remission, time to relapse, or disease-free periods [119].
Although cyclophosphamide and corticosteroids are effective in treating ANCA-associated vasculitis patients with mild to moderate disease, patients suffering from life-threatening vasculitis with organ failure require a more aggressive therapeutic regimen. In the MEPEX (intravenous methyl prednisolone versus plasma exchange as adjunctive treatment) study, performed by the EUVAS, patients with severe renal involvement (serum creatinine > 500 μmol/L) were treated with a standard therapy of cyclophosphamide and corticosteroids, in addition of which they received adjunctive treatment consisting of either intravenous methyl prednisolone or plasma exchange. Although a similar percentage of patients died in both groups (about 25% within the first year after diagnosis), plasma exchange proved to be superior to intravenous methyl prednisolone as adjunct to standard therapy with regard to dialysis-free survival after one year (80% versus 57%, respectively). In this thesis, renal histology and clinical aspects of this particular patient group with severe renal disease are studied in detail in chapter 2, 3, and 4.

Antibody therapy
Etanercept, which inhibits tumor necrosis factor alpha (TNFα), was studied by the Wegener's Granulomatosis Etanercept Trial (WGET) Group [120]. No beneficial effect was seen with the addition of etanercept, but there was a threefold increase in solid malignancies [121]. Infliximab, another anti-TNFα biological, has proved to be efficient in suppressing disease activity in a small number of patients with refractory vasculitis [122], however another pilot study contradicts this beneficial effect [123]. For patients with persistent disease activity, a single course of intravenous immunoglobulin (IVIg) treatment (2 g/kg) was proven to be effective in reducing disease activity, although the effect was of short duration [124]. The immunosuppressant gusperimus (deoxyspergualin), which inhibits growth of activated naïve CD4+ T-cells, has also been shown to

have a high level of efficacy in remission induction in refractory or relapsing Wegener's granulomatosis [125]. Rituximab, a B-cell depleting drug targeted against the B-cell surface antigen CD20, has been effective in patients with active severe ANCA-associated vasculitis not tolerating or not responding to cyclophosphamide treatment [126]. Long term analysis of rituximab treatment is promising: in patients with chronically relapsing WG it prohibited relapse as long as B-lymphocytes were undetectable, and long term B cell depletion was well tolerated [127;128]. With two randomized controlled clinical trials underway studying the effects of rituximab in ANCA-associated vasculitis, this might be a promising drug for the future [125].

An international vasculitis network: EUVAS

Since ANCA-associated vasculitis is a rare disease, for performing clinical trials in uniform groups of patients that are large enough to make statistically significant conclusions, there is a need for investigators to work together. This led to the initiative of founding a group, later known as the European Vasculitis Study Group (EUVAS) in 1993. Its aim was to standardize current treatment regimens and test new treatment regimens. Physicians with an interest in the field of ANCA-associated vasculitis joined efforts to start a number of multicenter randomized controlled clinical trials. To harmonize these clinical trials, validated scoring systems were created for both clinical manifestation [129-131] and histology [132]. The latter system was used in the studies described in chapter 2, 3, and 4 in this thesis. Within the EUVAS, efforts have been joined to standardize the analysis of serologic ANCA [39;133;134]. The EUVAS stimulates and facilitates top-level scientific and clinical collaboration concerning primary ANCA-associated vasculitis. Most of the work, described in this thesis originated from this collaborative group.

Outline of the thesis

This thesis commences with a focus on the patients with ANCA-associated vasculitis presenting with rapidly progressive renal disease in chapter 2. Clinical and histological determinants of renal and patient outcome one year after diagnosis are extensively described. Hereafter, attention is focused on patients who present with dialysis dependent ANCA-associated glomerulonephritis in chapter 3. Treatment decisions are discussed, taking into account the chance of recovery and that of dying from therapy. Both patients with mild to moderate and severe renal involvement at diagnosis were followed for five years, after

which long term outcome was analyzed. For these patients chances of recovery, dialysis dependency, and death were studied. Predictors of five-year outcome are extensively described in chapter 4. Moreover, a model is proposed to calculate the chance on each of these outcomes after one year and after five years.
An overview of the most common hypotheses on the etiology of ANCA-associated vasculitis is given, in chapter 6. Many potential initiating factors have been launched over the past two decades, but no single factor has been proven to be exclusively responsible for the induction of vasculitic disease. Most current views explain the disease by a combination of different environmental factors that superimpose upon a genetic susceptibility for developing this disease. Finally, the results of all these studies are summarized and more extensively discussed in chapter 7.

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