

# New treatments of ANCA-associated vasculitis: An overview

Ammerens Menage\*

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## Abstract

We provide an overview of anti-neutrophil cytoplasmatic auto antibodies (ANCA)-associated vasculitis, in which we present the most recent information regarding different forms of vasculitis, with a specific focus on ANCA-associated small vessel vasculitis. After examining the role of ANCA in the development of ANCA-associated vasculitides, the standard treatment strategies and a number of new possible treatments—rituximab, TNF- $\alpha$  antagonists, and endoglycosidase—are detailed, along with their benefits and drawbacks. We argue that rituximab is a suitable alternative to standard therapy, while studies and treatments involving TNF- $\alpha$  antagonists, especially etanercept, seem less convincing. Although research into the workings and effects of endoglycosidase are still at an early stage, using EndoS may well be an effective new strategy in treating ANCA-associated vasculitis and other (auto) antibody-mediated diseases.

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\*Student ID 1736000, e-mail: A.Menage@student.rug.nl. Supervisor: dr. M.M. Van Timmeren, University of Groningen, University Medical Center Groningen, Medical Biology Division, Endothelial Biomedicine & Vascular Drug Targeting Group, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. The author thanks dr. Van Timmeren for fruitful discussions and comments on a previous draft.

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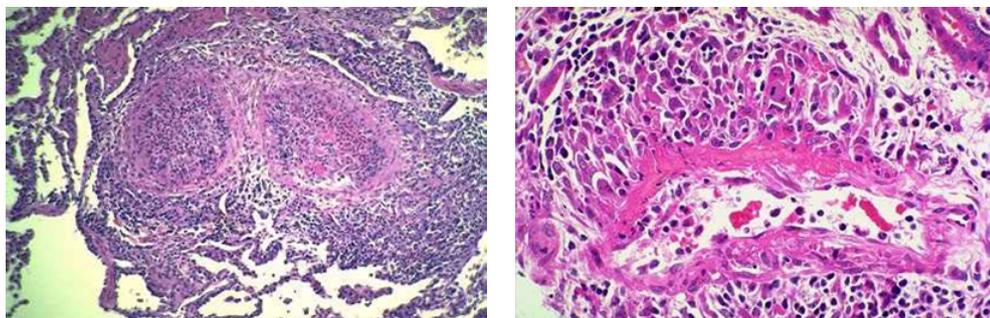
# 1 Introduction

This paper presents a systemic overview of the auto-immune disease called anti-neutrophil cytoplasmatic auto antibodies (ANCA)-associated vasculitis, in which we discuss what is currently known about this disease and provide a detailed review of current and possible future treatments. This paper is structured as follows. In Section 2, we outline the most recent information regarding the different forms of vasculitis, with a particular focus on small vessel vasculitis and an examination of the role of ANCA in the development of ANCA-associated vasculitides. Section 3 presents the standard treatment strategies and discusses their benefits and shortcomings, followed by a discussion of new possible treatments. The perspectives for these treatments are discussed in Section 4. Section 5 concludes.

## 2 Vasculitides

### 2.1 Systemic vasculitides

Vasculitis is a non-specific term for many different disorders; they are all defined by inflammation of the vessel walls in which leukocytes are present (Figure 1). Any of the body's blood vessels can be affected, including arteries, arterioles, capillaries, venules and veins.



(a) Necrotizing and granulomatous vasculitis in the lung of a patient with Churg-Strauss syndrome.

(b) Small-vessel necrotizing vasculitis.

Figure 1: Histological examples of different forms of vasculitis. *Source:* Jennette (1997).

There are various forms of vasculitis and the pathogenesises of these diseases are not yet completely understood. Some vasculitides cause systemic inflammation in multiple sites in the human body, whereas other types only cause vessel inflammation in specific organs. Because different sites of the

vascular system can be affected, vasculitis presents itself in many different forms. Inflammation of the vessel wall may lead to increased permeability, stenosis, aneurysms formation, hemorrhage and formation of thrombosis (Langford, 2010). Inflammation often leads to necrosis of the blood vessel walls and sometimes even to necrosis of surrounding tissue, often resulting in dangerous conditions.

Although the causes of vasculitis are not yet fully known, vasculitides are generally divided into two groups: primary and secondary vasculitis. Primary vasculitis involves an unknown cause of the onset of the inflammation. Secondary vasculitis is provoked by an infection, a toxin, a drug or takes place as part of another inflammatory disorder or cancer (Fries et al., 1990; Langford, 2010).

## 2.2 Classification

Kussmaul and Maier were the first to describe a form of vasculitis called polyarteritis nodosa. Their description of the affected arteries is “cord-like arteries with frequent nodular protrusions” (Kussmaul and Maier, 1866). Due to the increased use of light microscopy, it was found that not only arteries but also venules, capillaries and small arterioles are sensitive to vasculitis. Wegener described vasculitis involved in the arterial system and the kidneys, which later became known as Wegener’s disease (Wegener, 1939).

Over the years, many different forms of vasculitis have been discovered, resulting in various possibilities to classify them. The American College of Rheumatology has classified vasculitis based on data derived from patients with fully developed classic diseases, such as histopathological findings and clinical symptoms. Definitions for various vasculitides based on the size of the vessels that are involved have been proposed at the International Consensus Conference in 1994 (Figure 2). Vasculitis in the large vessels includes diseases such as Takayasu’s arthritis and Giant cell (temporal) arthritis. Medium sized-vessel vasculitis includes polyarteritis nodosa and Kawasaki’s disease. Small-vessel vasculitis includes microscopic polyangiitis, Wegener’s granulomatosis, essential cryoglobulinemic purpura and Churg-Strauss syndrome (allergic granulomatous angiitis) (Jenette et al., 1994).

The pathogenesis of the diverse vasculitides varies. In large vessels, such as the aorta, inflammation occurs in numeral disorders which share their pathogenic pathways. A cellular immune response takes place, involving antigen-presenting cells, macrophages and T cells. Medium and small vessel vasculitis differ in their pathogenesis. Some of them are characterized by high levels of immune complexes, e.g. Henoch-Schonlein purpura is accompanied by high levels of immunoglobuline A (Figure 3a). Another group of vasculitides is called pauci-immune vasculitides, which are characterized by the presence of auto antibodies against neutrophil cytoplasmatic components, known as anti-neutrophil cytoplasmatic auto antibodies (ANCA).

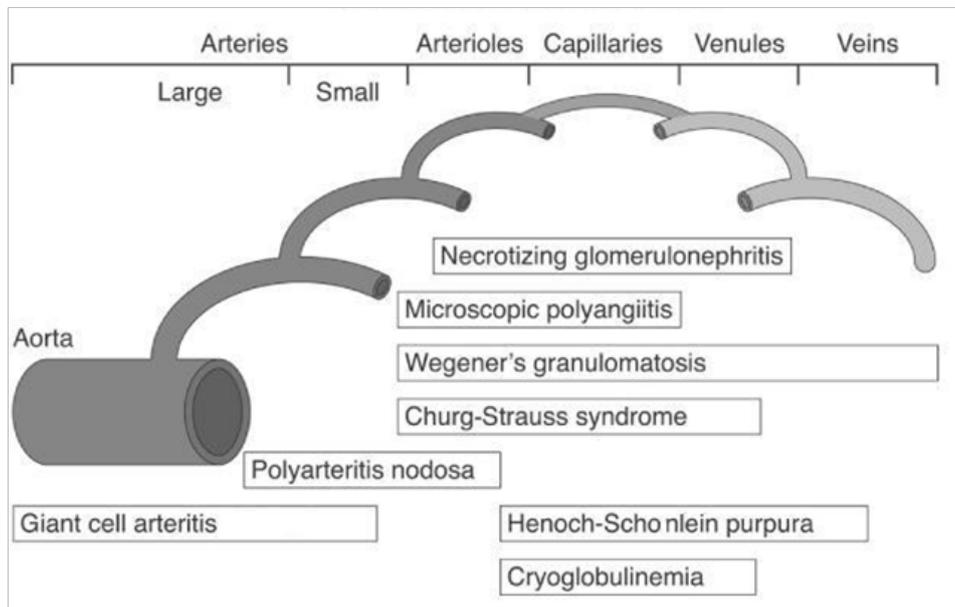


Figure 2: Classification of the systemic vasculitides. *Source:* Jenette et al. (1994).

The amount of immunoglobulin deposition at the site of the inflammation in pauci-immune vasculitides is small. An example of ANCA-associated vasculitis is Churg-Strauss syndrome (Figure 3b) (Kallenberg et al., 1994). The remainder of this paper focuses on a specific group of vasculitides—small vessel vasculitis—which is associated with the presence of ANCA (Weyand and Goronzy, 2003).

## 2.3 ANCA-associated vasculitis

Diseases involving ANCA-associated vasculitides are: Wegener's granulomatosis (WG), Churg-Strauss syndrome (CSS) and microscopic polyangiitis (MPA). These are progressive inflammatory autoimmune diseases mainly affecting small blood vessels (Morgan et al., 2006). Neutrophils infiltrate the capillaries, arterioles and venules in the skin and kidneys and in the alveoli of the lungs, leading to widespread hemorrhage. This subsection presents an overview of these diseases and discusses their histopathological findings and ANCA levels.

### 2.3.1 Histopathological findings

Wegener's disease often involves the upper and lower airways. Pulmonary symptoms are found in 60-80 percent of patients. The patients' most com-



(a) Purpura caused by SVV, in (b) Skin rashes caused by CSS. this case Henoch-Schonlein.

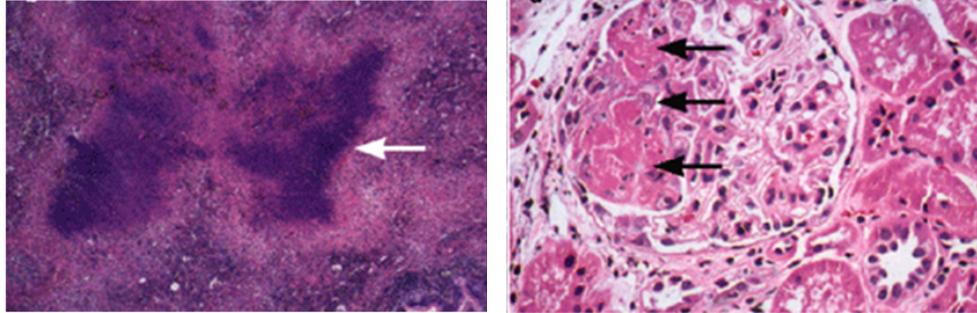
Figure 3: Histological examples of different forms of vasculitis. *Source:* Farid-Moayer and Lowe (2009).

mon manifestations are coughing up of blood or bloody sputum (hemoptysis), pulmonary hemorrhage and inflammation of the pleura (pleuritis). Histological features of lung biopsies from patients regularly show granulomatous inflammation and necrosis (Figure 4a).

The kidneys are also involved with Wegener's granulomatosis. Renal involvement is mostly manifested by acute renal failure. Renal illness is diagnosed in 18 percent of patients at the outset of the disease, but 77 percent develop glomerulonephritis at a later stage (Fauci et al., 1983; Hoffman et al., 1992). Microscopic polyangiitis shares many characteristics with WG. The kidneys are perpetually affected and the pulmonary tract is also often involved. MPA and WG have comparable features on renal histology, for example a focal necrotizing and pauci-immune glomerulonephritis (Savage et al., 1985).

In patients with WG or MPA, circulating ANCA leads to inflammation in the kidneys. Histological images of the kidney show an infiltrate of inflammatory neutrophils, focal and necrotizing, crescentic glomerulonephritis and segmental glomerular necrosis reflecting a glomerular capillaritis (Figure 4b). This is commonly known as necrotizing and crescentic glomerulonephritis (NCGN) (Hauer et al., 2002). Rapidly progressive glomerulonephritis and the progression to the following end-stage of renal failure are caused by vasculitis in 80 percent of the cases (Jayne et al., 1990; Falk et al., 2000).

Churg-Strauss syndrome mainly involves the blood vessels in the lungs. The granulomas in the lungs in CSS, also called allergic granulomas, typi-



(a) The arrow points at granulomatous and necrosis in the lung tissue. (b) The arrows indicate early crescent formation in the kidney's glomeruli.

Figure 4: Biopsies from WG patients. *Source:* Sarraf and Sneller (2005).

cally involves borders of keratin, histiocytes (macrophages) and multinucleated giant cells surrounding a central necrotic zone consisting of the necrotic eosinophils (Figure 5 and 1a). CSS often starts with severe asthma with 97 percent of patients, later affecting the gastrointestinal tract, cardiovascular system and the skin's blood vessels (Figure 3b). Peripheral nerves are affected more severely under CSS compared to MPA and WG, but renal disease occurs less frequently, while NCGC is most commonly found.

### 2.3.2 Myeloperoxidase and proteinase 3

ANCA-associated vasculitis is typically characterized by the presence of ANCA. The two most common forms of ANCA are myeloperoxidase (MPO) and proteinase 3 (Pr3), which are associated with different forms of pauci-immune vasculitis. Anti-myeloperoxidase antibodies (MPO-ANCA) are predominantly found in MPA and CSS, whereas ANCA directed to proteinase 3 (PR3-ANCA) are detected mainly in WG. Both PR3 and MPO are situated in the peroxidase-positive lysosomes of monocytes and in the azurophilic granules of neutrophils.

Special tests have been developed to detect and illustrate the presence of ANCA. First, immunofluorescence staining of ethanol-fixed neutrophils is used to detect the cytoplasmic pattern of c-ANCA or the perinuclear pattern of p-ANCA. Sera containing PR3-ANCA cause a cytoplasmic pattern of neutrophil staining, a c-ANCA pattern. Sera containing MPO-ANCA, however, lead to a perinuclear, p-ANCA pattern of staining. After immunofluorescence staining, enzyme-linked immunosorbent assay (ELISA) is used. ELISA is used to check for antibodies specific to the autoantigens PR3 and MPO, to determine whether these correlate with the c- and p-ANCA patterns (The Merck Manual, 2005). See Figure 6.

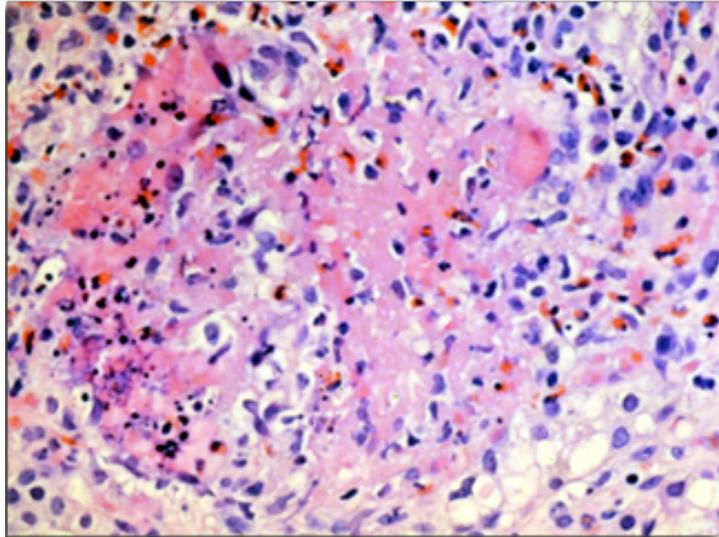


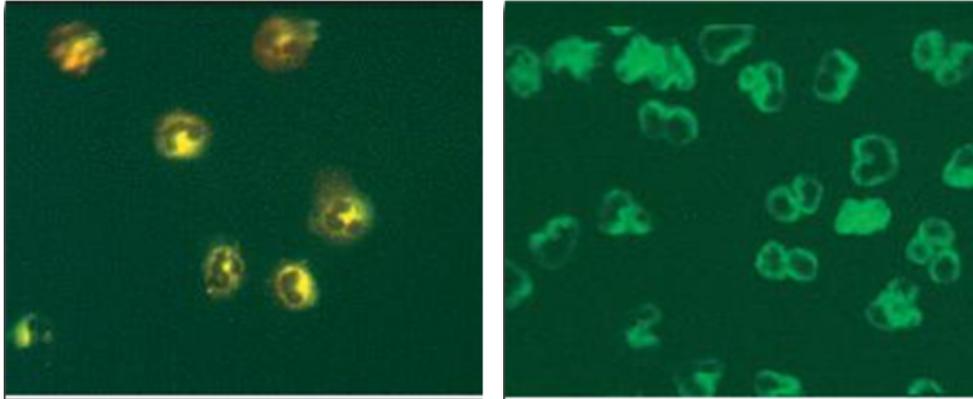
Figure 5: Allergic granuloma in a CSS patient. *Source:* Hernandez (2009).

More than 90 percent of patients with active Wegener's granulomatosis have ANCA in the serum (Wiik, 2009). Most patients with Wegener's granulomatosis have c-ANCA, characterized by autoantibodies directed against PR3; only 10 percent have p-ANCA directed against MPO. Approximately 60 percent microscopic polyangiitis patients are ANCA positive, mainly involving MPO-ANCA. Only a few patients have PR3-ANCA (Guillevin et al., 1999). In Churg-Strauss patients, MPO-ANCA are detected in approximately 40 to 60 percent, while less than 5 percent have PR3-ANCA (Conron and Beynon, 2000). In idiopathic crescentic glomerulonephritis, 64 percent of the patients show ANCA-MPO and 30 percent show PR3-ANCA. Pauci-immune small vessel vasculitis (SVV) is very similar to ANCA-associated small vessel vasculitis, though it should be noted that some pauci-immune SVV patients are ANCA negative.

#### 2.4 Are ANCAs pathogenic?

The pathogenesis of progressive inflammatory ANCA-associated vasculitis still has many unanswered questions. The presence of anti-MPO antibodies boosts the inflammatory reaction, probably because they can activate neutrophils and start an autoimmune response. Activation of these neutrophils leads to an increased inflammatory response and adhesion to endothelial cells, causing endothelial cell damage (Falk and Jennette, 2010). But what proves the pathogenicity of PR3-ANCA and MPO-ANCA?

The direct pathogenic role of MPO-ANCA and PR3-ANCA is observed in several *in vitro* and *in vivo* studies. The interaction of ANCA with neu-



(a) Stained neutrophils of an active Wegener's patient with PR3-ANCA, showing a granular, p-ANCA. (b) Sample of patient with ANCA-MPO, which shows a perinuclear staining pattern, p-ANCA. c-ANCA, fluorescent pattern.

Figure 6: Examples of ANCA after immunofluorescence staining. *Source:* Kallenberg et al. (2006).

neutrophils and endothelial cells causes endothelial damage. After activation by, for example  $\text{TNF-}\alpha$ , neutrophils degranulate and express MPO and PR3 on their surface and release proinflammatory molecules such as oxygen radicals, cytokines and other enzymes. This process is demonstrated in various *in vitro* studies (Heeringa et al., 1996; Kallenberg et al., 2006; Falk et al., 1990). *In vitro* studies show that neutrophil activation by ANCA is mediated by the FC-receptor and the binding of ANCA Fab'2 to the antigens at the surface of neutrophils (Mulder et al., 1994).

There is also evidence that MPO-ANCA has a pathogenic effect *in vivo*. In the past decades, several animal models have been introduced and developed, but all of them have had limitations. Xiao et al. (2002, 2005) have been able to develop a mouse model which provides direct and convincing evidence that MPO-ANCA can cause pauci-immune glomerulonephritis and vasculitis in mice. By immunization of MPO-deficient mice with murine MPO, this leads to the development of antimurine MPO antibodies. NCGN is then induced in approximately 80 percent of the glomeruli by passive transfer of splenocytes, including anti-MPO B cells, from MPO $-/-$  mice that have been immunized with murine MPO in Rag2 $-/-$  immune deficient mice (Figure 7). Xiao et al. (2005) report that the development of NCGN in this experimental model is accompanied by glomerular accumulation of neutrophils and macrophages. When they depleted neutrophils with NIMP-R14 rat monoclonal antibodies from the circulation, this resulted in complete protection from anti-MPO IgG-induced NCGN. These findings point to a very important role of neutrophils in the pathogenesis of NCGN.

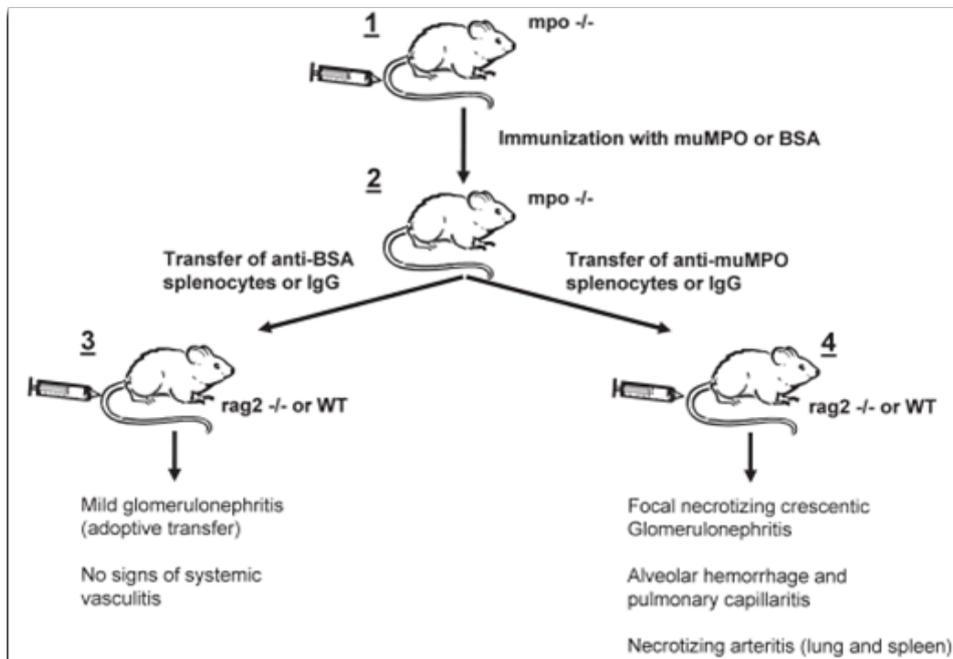


Figure 7: Strategy for inducing a mouse model of anti-myeloperoxidase (MPO)-associated glomerulonephritis and vasculitis, including pulmonary capillaritis. 1. Myeloperoxidase-deficient mice (MPO<sup>-/-</sup>) are immunized with murine MPO (muMPO) or bovine serum albumin (BSA). 2. Splenocytes or immunoglobulin G (IgG) are obtained from immunized MPO<sup>-/-</sup> mice. 3. Adoptive transfer of BSA+ splenocytes into RAG2<sup>-/-</sup> or passive transfer of BSA+ IgG into RAG2<sup>-/-</sup> or wild type mice induces no disease. 4. Adoptive transfer of muMPO+ splenocytes into RAG2<sup>-/-</sup> or passive transfer of muMPO+ IgG into RAG2<sup>-/-</sup> or wild type mice induces vasculitis and necrotizing crescentic glomerulonephritis. *Source:* Travis (2004).

The pathogenic role of PR3-ANCA is found in *in vitro* studies, but not in *in vivo* experiments. However, WG patients have elevated proportions of membrane PR3-positive neutrophils that are associated with an increased frequency of relapse (Witko-Sarsat et al., 1999; Schreiber et al., 2003).

#### 2.4.1 ANCA's pathogenic effect

Although the pathogenic effect of ANCA on vasculitis is not fully known, several elements have been identified that can be associated with the mechanism of these diseases. The pathogenic role of ANCA is supported by a number of findings. First, two drugs, propylthiouracil and hydralazine, stimulate ANCA and necrotizing glomerulonephritis and vasculitis (Peacock and Weatherall, 1981; Griswold et al., 1978). Second, increased ANCA titers can indicate sickness relapses (Boomsma et al., 2000). Third, a new-

born developed glomerulonephritis after birth from a mother with MPO-ANCA-associated polyangiitis. The newborn apparently developed glomerulonephritis by transferred MPO-ANCA (Schlieben et al., 2005; Bansal and Tobin, 2004). This implies that ANCA has a pathogenic role.

#### 2.4.2 Current understanding of the pathogenesis

The current understanding regarding the pathogenesis of ANCA-associated small vessel vasculitis is that neutrophils are primed by cytokines and chemokines, from a local or systemic infection or from the expression of endothelial adhesion molecules (see Figure 8). This priming results in the up regulation of the expression of neutrophil adhesion molecules and the translocation of the ANCA antigens to the cell surface. The dimers of the antigen-binding fragment of ANCA IgG ( $F(ab')_2$ ) bind with ANCA antigens on the membrane. Now the ANCA Fc tail binds to expressed  $Fc\gamma$  receptors ( $Fc\gamma R$ ),  $Fc\gamma RIIa$ , and  $Fc\gamma RIIIb$  (Falk et al., 1990; Heeringa et al., 2005; Mulder et al., 1994; Porges et al., 1994). These bindings activate neutrophils and lead to increased transmigration and adherence of neutrophils to vessel walls. The ANCA-mediated neutrophil activation also provokes production of reactive oxygen radicals and leads to possible neutrophil degranulation. The release of proteolytic enzymes leads to a serious inflammation throughout the vessel wall, called vasculitis. The inflammation may eventually lead to organ damage (reviewed by Van Timmeren et al. (2009)).

### 2.5 Origin of ANCA

Which factors contribute to the formation of ANCA, how are they produced, and why? There are several hypotheses about the initiating factor of ANCA production. The first factor is exposure to silica in the environment; this often leads to ANCA-associated glomerulonephritis (Gregorini et al., 1997). Secondly, microbial infections such as *S.aureus*, *S. maltophilia* and *H. influenzae* have been linked with the beginning of ANCA-associated systemic vasculitis. The presence of lipopolysaccharide (LPS) on the outer membrane of these bacteria probably leads to inflammation. The infections are easily treatable with antibiotics to prevent e.g. relapses (Park et al., 2004; Boudewyns et al., 2001). Genetic predisposition is the third hypothesis. This is a broadly accepted theory because there are several described familial cases of ANCA-associated vasculitis (Nowack et al., 1999). Using the thyroid drug propylthiouracil is the fourth cause of increasing ANCA levels, which can lead to vasculitis (Dolman et al., 1993). The last hypothesis regarding the onset of ANCA-associated vasculitis is the parvovirus B19, although supporting evidence is not yet convincing (Finkel et al., 1994).

The next question that comes to mind is: what is the underlying cause of the immune response against ANCA? Until now, three theories have been

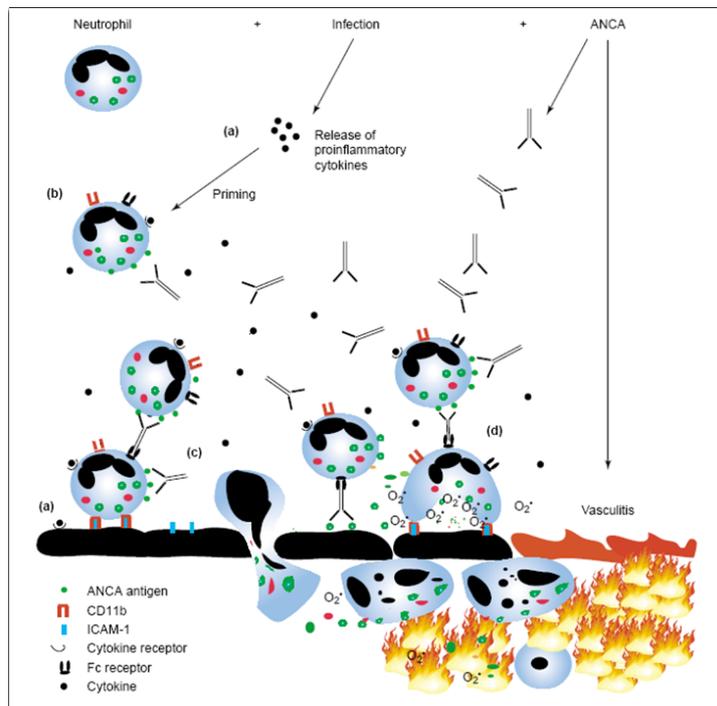


Figure 8: Representation of ANCA-mediated neutrophil responses that are putatively involved in the pathogenesis of ANCA-associated small vessel vasculitis. (a) Neutrophils are primed by cytokines and chemokines (e.g. tumor necrosis factor). (b) By priming the up regulation of the expression of neutrophil adhesion molecules and the translocation of the ANCA antigens to the cell surface is induced. (c) Activated neutrophils cause increased transmigration and adherence of neutrophils to vessel walls. (d) Activated ANCA-mediated neutrophils release oxygen radicals and possibly causes neutrophil degranulation. Proteolytic enzymes, released by this process, can lead to vasculitis. *Source:* Heeringa et al. (2005).

put forward about the origin of the ANCA immune response. The first theory is that neutrophils may be surrounded by neutrophil NETs, which leads to an autoimmune response due to a high content of PR3 and MPO (Kessenbrock et al., 2009). The second theory, autoantigen complementary, assumes that a protein complementary to human autoantigen PR3 triggers an ANCA immune response (Pendergraft et al., 2004). The third, and until now, last theory regarding the genesis of ANCA disease is the association with antibodies to lysosome-associated membrane protein 2 (LAMP-2). In patients with both PR3-ANCA and MPO-ANCA, it has been observed that they have antibodies to LAMP-2. *In vitro* it has been showed that LAMP-2 is capable of causing endothelial damage and neutrophil activation. Antibodies to LAMP-2 peptides are also capable of inducing NCGN in rats.

This theory, however, still has to be confirmed (Kain et al., 2008; Falk and Jennette, 2010).

## 2.6 Summary

Vasculitis is a severe disease and sometimes even fatal. It requires punctual recognition and treatment. Symptomatic involvement of affected organs may take place in isolation or in combination with multiple organ involvement, e.g. renal failure, pulmonary infiltrates, skin rashes, neurological manifestations such as peripheral neuropathy or constitutional symptoms (Seo and Stone, 2004; Mansi et al., 2002; Jennette and Falk, 1997).

Progression of ANCA-associated vasculitis, e.g. glomerulonephritis, can be prevented by immunosuppressive and steroid therapy (Booth et al., 2003). However, current treatments are associated with adverse effects, without preventing the occurrence of relapses. Therefore, more effective and less toxic therapies are needed.

The remainder of this paper is structured as follows. First, we identify the present treatments of ANCA-associated vasculitis and discuss their benefits and shortcomings. We then turn to an overview of a discussion of new possible medicines and treatment strategies, followed by a discussion of the most viable options for treatment and future research.

## 3 Theories and Results

Until the late 1970s, ANCA-associated small vessel vasculitis (SVV) was fatal with 80 percent of patients surviving less than two years. However, patients' life expectancy has increased significantly since the introduction of cyclophosphamide (CYC) and the immunosuppressor prednisolone. In a study of 246 patients with ANCA-associated vasculitis and renal involvement, the 5-year mortality was 76 percent. The cause of death, however, is often infection (50 percent) in the first year after diagnosis. Active vasculitis itself causes 14 percent of deaths. In prolonged immunosuppressive therapy, many patients also die due to an infection. This reinforces the need for improved long-term immunosuppressive therapy or other new treatments (Booth et al., 2003; Mukhtyar et al., 2008; Turnbull and Harper, 2009).

### 3.1 Standard treatment strategies

The treatment of ANCA-associated SVV is divided into several phases. The initial phase is aimed at inducing remission and the second phase at maintaining the remission and preventing relapse. A combination of prednisolone and cyclophosphamide is used in standard induction therapy, inducing remission in up to 90 percent of the patients. In less severe cases cyclophosphamide is often replaced by the drug methotrexate. Maintenance therapy

is able to limit the rate of relapses to 18-40 percent, cyclophosphamide is then often replaced by azathioprine or methotrexate (Jayne et al., 2003; De Groot et al., 2005; Ozaki, 2007). In extreme cases of ANCA-associated SVV, plasma exchange is used. But how do these drugs work and what are their side effects?

### **3.1.1 Cyclophosphamide**

Cyclophosphamide is a pro-drug that must be metabolized in the liver to be active. It is often used as an immunosuppressor. It is an alkylating drug that inhibits division of cells, leading to programmed cell death. The use of cyclophosphamide often results in lymphopenia, particularly of B cells and results in the suppression of humoral responses (Mukhtyar et al., 2008; Hall and Tilby, 1992; Up To Date, 2010a).

Unfortunately, patients using cyclophosphamide may suffer additional adverse effects. After starting the treatment, nausea and vomiting usually occur after 6-10 hours and diarrhea accompanied by other gastrointestinal side effects. Alopecia (hair loss) occurs in 40 to 60 percent of the cases. Hair loss generally begins 3-6 weeks after the therapy has been started. Hair growth generally returns to normal after the patient stopped using cyclophosphamide, though it may be a different color and/or texture. Endocrine and metabolic functions are also often disturbed. The drug interferes with oogenesis and spermatogenesis; this can be irreversible in some patients and leads to sterility. In women it often leads to amenorrhea, ovarian failure occurs in 57 percent of previously menstruating women (Hoffman et al., 1992; Watson et al., 1985). The urinary tract also suffers from side effects. Severe, potentially fatal acute hemorrhagic cystitis develops in 7-40 percent of the users. It may also lead to bladder cancer (Talar-Williams et al., 1996).

### **3.1.2 Prednisolone**

Prednisolone is an anti-inflammatory agent in the group of corticosteroids. It decreases inflammation by suppressing migration of polymorphonuclear leukocytes and reversal of augmented capillary permeability. Prednisolone suppresses the immune system by reducing the activity and volume of the lymphatic system; this decreases the harmful response of the body to the disease.

Unfortunately, prednisolone causes unwanted side effects. Patients using prednisolone have a high risk of infection, which is an important cause of death. Bacterial infections are, however, usually easily treated with antibiotics. A high dose of prednisolone, especially in the initial phase, leads to metabolic changes. In the first year the frequency of steroid-induced diabetes was 8 percent, whereas, overall, 9.4 in 100 patients became diabetic. Other metabolic changes lead to hypertension, hyperglycaemia, fluid retention and,

in the long run, serious consequences for the cardiovascular system (Hoffman et al., 1992). The hypothalamic-pituitary-adrenal axis is also frequently affected through corticosteroid use. Patients experience adrenal insufficiency and weight gain which contributes to the risk of cardiovascular disease. 1 in every 5 patient gained more than 10kg and maintained that weight for at least 1 year (Domsic et al., 2006; Wung et al., 2008). The central nervous system is also often involved. Side effects like vertigo, seizure, headache and nervousness take place. Psychiatric effects such as depression and mania occur with doses above 40mg per day. Severe reactions occur in 5 percent of the treated patients. When prednisolone is used in chronic treatment, it results in osteoporosis. This is because the corticosteroids interfere with the osteoblast process, hormonal axes and calcium homeostasis, leading to weakened bones (Clowes et al., 2001).

### **3.2 Plasma exchange therapy**

When severe vasculitis is presented in patients including renal failure and serum creatine  $> 500\mu\text{mol/L}$ , additional plasma exchange therapy can be used. Plasmapheresis is a blood purification procedure that can be used to remove antibodies from the bloodstream. Blood is removed from the bloodstream, after which blood cells are separated from plasma. The blood is then diluted with fresh plasma or a substitute and returned into the bloodstream. An important advantage of this technique is the ability to remove harmful antibodies and proinflammatory factors from the patient's blood. However, it is a temporary benefit because the body still produces antibodies.

Jayne et al. (2007) study renal recovery when additional plasma exchange therapy has been used beside oral prednisolone and cyclophosphamide, followed by azathioprine for maintenance (De Lind Van Wijngaarden et al., 2006). The additional therapy resulted in better renal recovery compared to additional intravenous methylprednisolone. Plasma exchange therapy is a good alternative in ANCA-associated vasculitis that is presented with renal failure, especially with pulmonary hemorrhage (Klemmer et al., 2003). Plasmapheresis has some risks, involving anaphylaxis, a (severe) allergic reaction, and bacterial infection is also possible.

### **3.3 Challenges**

The key to reducing the burden of the existing therapies is to learn more about the diseases mechanisms. Prevention of the adverse effects that are associated with therapy will improve the outcome of new therapies, since 25 percent of patients suffer from severe adverse events such as infections and malignancy. Furthermore, the high rate of relapse, 50 percent, should be decreased to prevent accumulating damage from treatment and scars

(Booth et al., 2004; Hoffman et al., 1992). The medicines that are already in use, such as prednisolone, cyclophosphamide and azathioprine, have to be optimized to reduce therapy-related mortality and morbidity. Especially when remission is achieved, the use of immunosuppressants other than prednisolone may decrease adverse effects.

### 3.4 Rituximab

One of the new promising treatments of ANCA-associated vasculitis is the drug rituximab. Rituximab is a chimeric, monoclonal IgG1 autoantibody directed against CD20; it leads to the destruction of B cells via complement mediated lysis and antibody dependent cellular cytotoxicity. Rituximab leads to a quick depletion of circulating B cells for at least 6 months, making them undetectable in peripheral blood (Reff et al., 1994). CD20 is a transmembrane surface antigen of B cells, but the role of the antigen is not yet known. On plasma cells and pre-B cells the CD20 antigen is not expressed and thus these cells are not affected by the therapy.

B cell depletion with rituximab has proved effective in autoimmune diseases like systemic lupus erythematosus, rheumatoid arthritis and ANCA-associated vasculitis (Keogh et al., 2005). The drug rituximab was originally developed as an agent for the treatment of non-Hodgkin lymphoma (Maloney et al., 1997). A patient with both lymphoma and arthritis was treated with rituximab and showed a remarkable improvement: after three weeks he noticed improvement in joint pain and stiffness; three months later he was virtually symptom free (Protheroe et al., 1999). This incident sparked interest in using rituximab in anti-B cell therapy and several trials were set up for a variety of autoimmune diseases.

An auto-immune therapy directed towards B cells is a promising approach for several reasons. B cells are efficient antigen presenting cells; they activate cells that lead to cytokine production. Cytokines can have both pro- and anti-inflammatory effects. B cells themselves produce some pro-inflammatory cytokines, interleukin 6 (IL) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) (Mitchison, 2004) and can produce auto-antibodies. Patients with active vasculitis have a higher number of activated B cells compared to healthy people or patients in the remission phase (Popa et al., 1999). Rituximab decreased inflammation through the low level of activated B lymphocytes; they can no longer contribute to pro-inflammatory signals and processes.

Keogh et al. (2005) were one of the first to publish a study of 11 patients with ANCA-associated vasculitis to be treated with glucocorticoids and rituximab. The combination of glucocorticoids and rituximab has shown to give direct synergistic antiproliferative and apoptotic effects (Rose et al., 2002). All patients had been treated with maximum tolerated doses of cyclophosphamide and glucocorticoids, but this treatment did not improve the

patient's condition and remission was not achieved. The patients were then treated with rituximab and low doses of glucocorticoids. This gave promising results. First, all patients stayed in remission during B cell depletion, indicating that that B lymphocytes have a central role in the pathogenesis of ANCA-associated vasculitis (Cupps et al., 1982). Second, the trial emphasized the fact that the accumulation of ANCA is a predictor of relapse. All patients remained in remission, also after restoration of B cells (Boomsma et al., 2000). Keogh et al. (2005) conclude from their trial that either B lymphocytes or short-lived plasma cells (or both) are the cellular source of ANCA, because all the patients' ANCA titers decreased during treatment.

Rituximab therapy was tolerated well by all 11 users and unpleasant effects were uncommon, one patient suffered from dizziness, another from angioedema and three patients suffered from (mild) respiratory tract infections (Keogh et al., 2005). Although the negative effects seem to be minimal, they include fever, headache, rash, pruritus and angioedema. Abdominal pain and nausea also often occur. Moreover, the therapy results in temporary hematological changes such as cytopenias, lymphopenia, leucopenia, neutropenia and thrombocytopenia. Infusion-related side effects were observed in a few patients, being of mild intensity and not requiring discontinuation of treatment. Most of the adverse effects can be attributed to specific contributors and circumstances (Up To Date, 2010*c*).

B lymphocyte depletion may be a safe, effective therapy with few short-term side effects; effects on the long term have to be investigated. The peripheral B cell depletion leads to a fall in IgM serum levels and eliminates pathogens in the premature stages of B cell mediated (humoral) immunity before there is an excess level of IgG. IgG levels, however, were only marginally reduced, which may explain the small number of occurrences of serious infections.

Theoretically, there is a risk that users of rituximab develop antibodies against the chimeric, monoclonal IgG1 autoantibody, which are called human anti-chimeric anti-antibodies (HACA). Development of HACAs is an unpleasant side effect of rituximab; it could lead to infusion reactions or abrogate the effectiveness the next time the medicine is used. In a study in rheumatism arthritis patients, however, only 4 percent developed HACAs and no clinically adverse effects were noticed (Edwards et al., 2004; Flossmann et al., 2006).

Stasi et al. (2006) report their observations of a long-term study of ANCA-associated vasculitis patients who were treated with rituximab. All 10 patients experienced a fast clinical improvement due to the use of rituximab; in one patient, however, relapse occurred after six months. After 33.5 months three patients relapsed, but retreatment with rituximab again resulted in good responses. In all patients ANCA titers decreased significantly and only one had benign infusion-related side effects. They conclude that rituximab is an effective and well-tolerated treatment for patients with

ANCA-associated vasculitis and should be strongly considered for severely affected patients who do not respond to standard therapy or for those to whom cytotoxic therapy bears a high risk of morbidity.

Unfortunately, these results and conclusions were obtained from retrospective case series and small prospective uncontrolled studies. Next, we consider recent randomized, controlled trials to determine whether these promising results are consistent.

Stone et al. (2009) present a randomized controlled trial in which they investigate whether there is a difference in outcome between the use of rituximab or cyclophosphamide for induction of remission in ANCA-associated vasculitis. 197 patients were enrolled in the study who suffered from WG or MPA. For the induction of remission in severe ANCA-associated vasculitis, they found that rituximab is not inferior to cyclophosphamide. Adverse events were similar between groups, but fewer patients on rituximab experienced at least one adverse event. The authors conclude that rituximab is to be recommended as an alternative treatment.

Rituximab also shows promising effects as a maintenance therapy. These results come from a retrospective study with 39 patients where continuous use of anti-B cell therapy in patients with AAV in complete or partial remission is reported. The percentage of patients on cytotoxic immunosuppression or prednisolone decreased significantly. This extends the potential role of rituximab beyond induction to include maintenance therapy. However, more data are required regarding the delayed adverse effects of rituximab in randomized controlled trials (Rhee et al., 2010).

Rituximab seems to be a good complement in the standard therapy, but its role as a potential drug in ANCA-associated vasculitis and tool to help determine the role that B cells play in the disease requires further investigation.

### 3.5 TNF- $\alpha$ targeted medicines

One of the pro-inflammatory cytokines that B cells secrete is TNF. *In vitro* and *in vivo* experiments show evidence that TNF- $\alpha$  plays a central role in the pathogenesis of ANCA-associated vasculitis. TNF- $\alpha$  levels are increased in patients with active disease and are back to normal during remission (Noronha et al., 1993; Tesar et al., 1998). *In vitro* TNF- $\alpha$  and other cytokines are important for endothelial activation and neutrophil priming in ANCA-mediated vascular damage (Falk et al., 1990).

Two animal models show that anti-TNF- $\alpha$  antibodies can prevent and attenuate already established ANCA-induced NCGN (Huugen et al., 2005; Little et al., 2005). Inhibition of TNF- $\alpha$  could be an interesting drug target in different stages of ANCA-associated vasculitis. Several pilot and controlled studies of TNF- $\alpha$  blockade treatments have been performed with the drugs infliximab and etanercept.

The drug known as etanercept is a recombinant DNA-derived protein composed of TNF-receptor linked to the Fc portion of human IgG1. Etanercept binds predominately soluble TNF and blocks its interaction with cell surface receptors. The medicine infliximab is a high-affinity chimeric monoclonal anti-TNF- $\alpha$  antibody that blocks cytokine receptor ligation and neutralizes biological activity (Knight et al., 1993). Both drugs are used to treat diseases including Crohn's disease, psoriatic arthritis and rheumatoid arthritis (Up To Date, 2010b).

Like rituximab, the positive results in other autoimmune diseases led to the question whether these drugs are also effective in ANCA-associated vasculitis patients. The results of the different trials in patients with active ANCA-associated vasculitis are variable.

In a controlled trial with 180 WG patients, a combination of etanercept and CYC showed no benefit compared to other commonly used immunosuppressants. 6 patients (7 percent) in the etanercept group developed solid cancers within 2 years compared to 0 in the control group ( $p = 0.01$ ). The combination use of CYC and etanercept may increase the risk of cancers than the use of CYC alone. This led to the conclusion that etanercept is not effective for the maintenance of remission in patients with Wegener's granulomatosis (Stone et al., 2006; Wegener's Granulomatosis Etanercept Trial (WGET) Research Group, 2005).

A pilot study with 32 WG and MPA patients treated with additional infliximab gave moderately positive results but need to be confirmed in larger randomized trials. There were 2 deaths (6.7 percent) among patients with ANCA-MPO-associated renal vasculitis. One of the deaths was caused by diffuse pulmonary hemorrhage attributed to pulmonary vasculitis, the other caused by bronchopneumonia related with cyclophosphamide-induced leucopenia. Infliximab was successful at inducing remission in 88 percent of patients. Infections were seen in 21 percent of patients, and despite continued infliximab, 14 percent of initial responders experienced relapses after treatment (Booth et al., 2004).

### 3.6 IgG glycan hydrolysis

Rituximab and infliximab are both existing medicines that appear to have varying degrees of success in limiting the inflammatory reactions in ANCA-associated vasculitis and remission. Besides finding new applications of existing medicines, another approach is to develop an altogether new treatment that targets ANCA-associated vasculitis. Indeed, there is a need for a more directed treatment of ANCA-associated vasculitis to prevent total body immune suppression and other dangerous side effects. With increasing knowledge on the pathogenesis and effector mechanism of ANCA-associated vasculitis, new more specific treatments might be developed. Specifically, neutrophil activation leads to an augmented inflammation due to binding of

ANCA to ANCA antigens on the membrane of the neutrophil together with the binding of the ANCA Fc tail to the expressed Fc $\gamma$ R on the neutrophil membrane. One way to prevent neutrophil activation due to ANCA IgG is to incapacitate this connection. A number of promising experiments in this field are currently underway. Due to the scope of this paper, we focus on one such experiment involving endoglycosidase (EndoS).

### 3.6.1 EndoS

*Streptococcus pyogenes* is a human pathogen that can cause serious infections and invasive diseases (Cunningham, 2000). But can these bacteria only harm humans, or can they also be put to other uses? Hayano and Tanaka (1967) described that the bacteria *Streptococcus pyogenes* can release an enzyme that works on glycoproteins. The secreted enzyme, EndoS, is able to release the terminal sialic acid residues from the glycoprotein immunoglobulin G (IgG). We are interested in de IgG glycoprotein because Collin and Olsen (2001) showed that EndoS activity is specific for human IgG, while IgA and IgM were not affected.

IgG is a key player in the human immune response and also in ANCA-associated vasculitis. It has two identical light and heavy chains. The heavy chain contains one variable domain  $V_H$  and three constant domains ( $C_H1 - 3$ ). The light chain consists of one variable domain and a constant domain. The  $C_H2$  domain has a conserved  $N$ -glycosylation site at Asn297. A complex biantennary oligosaccharide is attached to the Asn297 site in human IgG. The oligosaccharide has an important functional relevance, as indicated by a number of studies. If the oligosaccharide has been removed from, e.g., murine IgG by hydrolyses, it can no longer activate complement, induce antibody-dependent cellular cytotoxicity or bind to Fc-receptors on macrophages (Figure 9). Moreover, deglycosylated IgG also diminishes the elimination of antibody-antigen complexes from the circulation (Nose and Wigzell, 1983; Collin and Olsen, 2001). IgG glycan hydrolysis might be an effective new strategy in treating (auto) antibody-mediated diseases (Collin et al., 2008).

### 3.6.2 Trials

*In vitro* treatment of mouse and human IgG subclasses with EndoS results in a severely reduced affinity to cellular Fc $\gamma$ Rs (Allhorn et al., 2008). In mice models of arthritis and immune thrombocytopenic purpura, pretreatment of the pathogenic auto-antibodies with EndoS abolishes development of the disease. Also in established immune thrombocytopenic purpura, systemic injection of EndoS has a protective effect on mice (Collin et al., 2008; Nandakumar et al., 2007). Albert et al. (2008) demonstrated that EndoS injection *in vivo* resulted in an efficient removal of the IgG-associated sugar

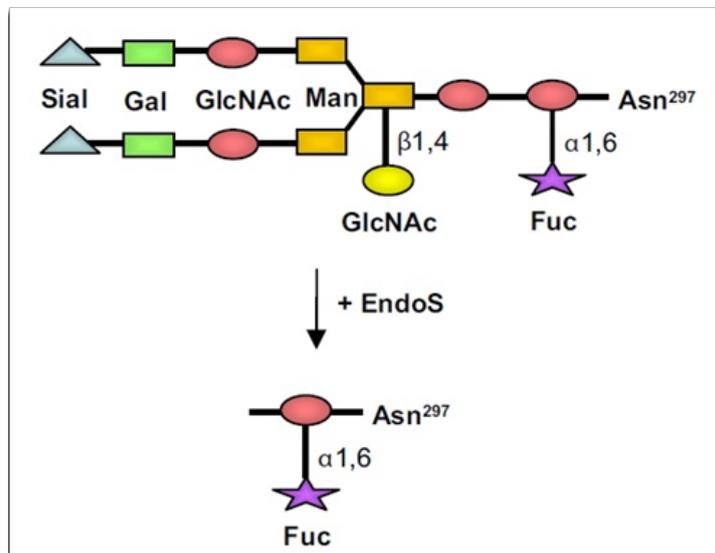


Figure 9: Hydrolysis of the IgG-associated glycan moiety by EndoS. Shown is the fully processed Asn-297 attached sugar moiety of IgG. *Source:* Albert et al. (2008).

domain, and interferes in various autoimmune models with autoantibody-mediated pro-inflammatory processes. After injection in mice, IgG completely becomes hydrolyzed and it stays in the mice's circulation for quite a few days. The therapeutic agent impairs the function of the IgG1 and IgG2b subclass *in vivo* in mice; other glycosylated proteins are not affected.

IgG glycan might also have a positive effect on ANCA-associated glomerulonephritis/vasculitis. Van Timmeren et al. (2010) hypothesize that Fc glycans of ANCA IgG are important in the development of the disease, therefore glycan hydrolysis by EndoS disables ANCA's pathogenic effect. To verify their hypothesis, the authors first investigated the effect of ANCA IgG-induced neutrophil activation *in vitro*. They isolated IgG from 11 ANCA positive patients and 5 controls. It was ruled out that the antigen-binding capacity of ANCA IgG is affected by EndoS treatment. Removal of the F(ab')<sub>2</sub> fragments of ANCA resulted in a disability to induce ANCA-mediated oxidative bursts. Deglycosylation of MPO- and Pr3-ANCA IgG by the use of EndoS resulted in less ANCA IgG-induced neutrophil degranulation, probably due to a strong attenuation of the neutrophil-activation capacity of ANCA IgG.

Next, Van Timmeren et al. (2010) tested whether the use of EndoS also had positive results *in vivo*. They used a mouse model of anti-MPO IgG/LPS-induced NCGN, in which an acute inflammation leading to NCGN was caused by co-administration of murine anti-MPO antibodies and LPS.

This model, however, is limited because the disease is passively induced and results in a rapid monophasic renal disease (Huugen et al., 2005). The results were quite promising. First, EndoS-mediated deglycosylation of anti-MPO IgG resulted in decreased urinary abnormalities. Second, it clearly reduced early glomerular neutrophil influx. Third, it practically avoids glomerular crescent formation in anti-MPO IgG/LPS-induced NCGN. Fourth, early (3 hours) systemic application of EndoS saved mice from disease progression.

The use of EndoS seems to be a promising therapeutic agent in ANCA-associated vasculitis, but there is a need for improved models to better mimic the true chronic and progressive nature of the disease. In addition, further research needs to be done to investigate concerns regarding toxicity in humans and a possible development of immunity against EndoS.

## 4 Discussion

We now turn to summarizing the medicines discussed in this paper and, in highlighting their main advantages and drawbacks, argue what we consider to be the most promising venues for further research.

The standard therapy of ANCA-associated vasculitis with cyclophosphamide and prednisolone has improved the outcome of ANCA-associated vasculitis. However, two important disadvantages of these treatments are their high relapse rates and side effects, including a high risk of developing infection, diabetes, leucopenia and malignancy due to nonspecific immunosuppressant. Renal failure caused by the disease increases toxicity, which is particularly problematic in case of MPO-ANCA related NCGN. In such cases, plasma exchange is a good additional treatment to reduce the damage to the kidneys and the need for future dialysis. To improve insight regarding patients with ANCA-associated vasculitis and standard therapy, the European League Against Rheumatism (EULAR) advises that patients should be treated by a group of ANCA specialists consisting of rheumatologists, nephrologists and specialists in internal medicine (Mukhtyar et al., 2008).

A number of new, promising drugs are available as alternatives to the standard therapy discussed above. One such a drug is rituximab. The results from the first trials are promising, although it should be noted that this medicine and other drugs were administered simultaneously, which may have influenced the results. Stone et al. (2009) show that rituximab can be used as an alternative treatment for ANCA-associated vasculitis, in that the drug leads to B cell depletion, which often leads to reduced inflammation and remission. Compared to cyclophosphamide, rituximab's advantage is that it is able to eliminate ANCA more directly. Rituximab in maintenance therapy also shows good results but needs additional research to better understand possible long-term effects. The exact working mechanism of rituximab is not fully understood yet, either. Rituximab may be a useful drug in performing

additional research to determine the exact role of B cells in general, and more specifically in the pathogenesis of ANCA-associated vasculitis. A number of studies are currently underway to shed light on these issues, especially with regard to the drug's long-term effects, including a randomized double-blind trial (RAVE) (see, e.g., Rose et al. (2010)). Overall, rituximab has received much attention from the research community and has gained much support as a viable alternative to standard therapy. Although it does not perform significantly better than standard treatment, it is a suitable alternative for certain patients who do not respond to standard treatment.

Another possible new treatment involves TNF- $\alpha$  antagonists. Compared to standard therapy, it has been found to be a more directed way to decrease endothelial activation and neutrophil priming in ANCA-mediated vascular damage. Studies with one such TNF- $\alpha$  antagonist, etanercept, did not prove to be more appropriate than standard therapy on account of 6 patients dying of severe side effects, all involving cancer. These results are sufficiently discouraging to render etanercept an unlikely candidate as an alternative treatment for ANCA-associated vasculitis. Of the TNF- $\alpha$  antagonists, only infliximab appeared to be promising in initial trials. However, treatments with infliximab present high rates of (severe) infection and as successful as other (standard and rituximab) treatments in causing remission. Moreover, confirmation is needed in randomized and long-term studies. So, although future research may provide new insights, the prospects seem limited.

Finally, a new strategy to interfere with the ANCA-mediated inflammatory process involves the drug EndoS. A major advantage is that the most potent pro-inflammatory subclass of IgG, that is crucial for the clearance of viral and bacterial infections, remains functional, also after EndoS treatment. EndoS, contrary to the known immunosuppressive drugs, will not impair the humoral defense mechanism completely. This might result in a decreased risk for the patients of having opportunistic infections, which are an important cause of death.

However, using glycan modification by EndoS as a treatment for ANCA-associated vasculitis needs further research in at least four areas. First, it must be examined how EndoS, a bacterial enzyme, can be safely administered to humans. Second, it must be determined that EndoS only affects glycans on human IgG, and not those on other proteins. Third, it remains to be established that EndoS discriminates between autoantibodies and naturally occurring protective antibodies so as not to compromise the immune defense system. Finally, studies have so far relied on mouse models with induced NCGN, while this condition is chronic and progressive in humans. Improvements to this model are needed to better mimic the disease. Overall, although this line of research may still be at an early stage, using EndoS may well be a promising treatment due to its specific working mechanism.

In arguing that there is considerable room for further studies in ANCA-associated vasculitis, we recommend that future clinical trials are performed

according to the EULAR's guidelines in order to further enhance transparency and consistency of studies and results.

## 5 Conclusion

We have provided an overview of ANCA-associated vasculitis, in which we presented the most recent information regarding different forms of vasculitis, with a particular focus on ANCA-associated small vessel vasculitis. After examining the role of ANCA in the development of ANCA-associated vasculitides, the standard treatment strategies and a number of new possible treatments—rituximab, TNF- $\alpha$  antagonists, and endoglycosidase—were discussed, along with their advantages and disadvantages. We have argued that rituximab is a suitable alternative to standard therapy, while studies and treatments involving TNF- $\alpha$  antagonists, especially etanercept, seem less promising. Although research into the workings and effects of endoglycosidase are still in their infancy, using EndoS may well be an effective new strategy in treating ANCA-associated vasculitis and other (auto) antibody-mediated diseases.

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