# Pathogenesis and treatment of ANCA-associated vasculitides

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**ABSTRACT**

*Recent studies have increased our insight into the pathogenesis of ANCA-associated vasculitis (AAV). Although many data from in vitro and in vivo experimental studies support the pathogenic role of the autoantibodies, cellular immunity seems involved as well. Besides, an amplification loop via the alternative pathway of complement is apparent. These new insights make a more targeted therapeutic approach possible. In particular, the B-cell depleting antibody rituximab has been shown non-inferior to cyclophosphamide for induction of remission, and even superior in patients with relapsing disease being positive for PR3-ANCA. Rituximab is also superior to cyclophosphamide for maintaining remission. Blocking the C5a-receptor seems promising as well as an alternative for high dose corticosteroids during induction of remission.*

## Introduction

The anti-neutrophil cytoplasmic autoantibodies (ANCA)-associated vasculitides (AAV) comprise granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) (1). These diseases are closely associated with ANCA, directed to proteinase 3 (PR3), particularly in patients with GPA, or to myeloperoxidase (MPO), particularly in patients with MPA. In EGPA, about 50% of patients are positive for ANCA, in most cases MPO-ANCA, and their clinical picture is dominated by small vessel vasculitis whereas clinical manifestations of the ANCA-negative subset are dominated by tissue infiltration with eosinophils (2). Clinical observations as well as *in vitro* and *in vivo* experimental studies strongly suggest that ANCA are involved in the pathogenesis of the associated diseases. Further insight into the pathogenetic pathways has led to more targeted treatment. In this review recent developments in pathogenesis and treatment of AAV will be discussed.

## Pathogenesis

As mentioned, ANCA are present in almost all patients with AAV (Table I) (3). Besides, levels of ANCA rise preceeding a relapse of AAV although the strength of this association differs between studies resulting in a positive likelihood ratio (LR) of only 2.84 (CI 1.65-4.90) and a negative LR of 0.49 (0.27-0.87) in a recent meta-analysis (4). Possibly, changes in epitope specificity (5) or epigenetic modifications of ANCA (6) may, besides levels of ANCA, influence their pathogenicity. The observation of transplacental transfer of MPO-ANCA inducing vasculitis in a neonate is of interest as well (7). So, clinical observations suggest that ANCA are involved in disease pathogenesis but are not conclusive. Interestingly, genome wide association studies showed that genetic associations are stronger with the autoantibodies than with the associated diseases, MPO-ANCA being associated with HLA-DQ and PR3-ANCA with HLA-DP and with PR3 itself and its anti-proteinase (α1-antitrypsin) (8). This, also, suggests that the autoantibodies are basic to the development of AAV.

*In vitro* studies have shown that ANCA are able to further activate primed neutrophils to the production of reactive oxygen species and the release of lytic enzymes. In the context of endothelial cells this leads to firm adhesion of neutrophils to the endothelium followed by endothelial cell detachment and lysis (summarised in 9). The alternative pathway of complement is involved as well in this process. Release of factor B, properdin and C3, a.o. from activated neutrophils, results in activation of the complement system with the generation of C5a which is a very strong chemoattractant for neutrophils and can also prime neutrophils. As such, complement activation, as a strong amplifi-

cation loop, seems to play an important role in ongoing neutrophil recruitment and activation (10). ANCA-induced neutrophil activation also stimulates the adaptive immune response, first by inducing neutrophil extracellular traps expressing PR3 and MPO on their surface so promoting the autoimmune response, and, secondly, by the release of B-cell activating factor (BAFF). So, ANCA-induced neutrophil activation appears to play a primary role in disease pathogenesis.

*In vivo* experimental studies strongly support a role for ANCA in disease development, particularly for MPO-ANCA. When splenocytes from MPO-deficient mice immunised with mouse MPO are transferred into normal recipient mice, these latter mice develop pauci-immune necrotising crescentic glomerulonephritis (NCGN) and haemorrhagic pulmonary capillaritis, completely congruent with MPA in humans. Transfer of IgG alone results in pauci-immune focal NCGN in the recipients. The alternative pathway of complement is involved in this model as factor B- and C5-deficient recipient mice do not develop disease and blockade of the C5a-receptor with an oral drug protects against the induction of MPO-ANCA NCGN. Also in a rat model of MPO-ANCA AAV, NCGN and pulmonary haemorrhage could be induced (summarised in 11). Development of an animal model for PR3-ANCA associated GPA has been less successful. Injecting human PR3-ANCA together with lipopolysaccharide in mice with a humanised immune system resulted in NCGN and pulmonary capillaritis in a minority of the mice but granulomatous inflammation, characteristic for human GPA, did not develop (12). So, *in vivo* experimental studies are strongly supportive for a pathogenic role of MPO-ANCA but not fully for PR3-ANCA.

Granulomatous inflammation as seen in GPA suggests involvement of cellular immunity. Indeed, effector memory CD4-positive T-cells appeared to be increased in the peripheral blood in GPA patients and could be demonstrated in the urine during active renal disease. In addition, the cytokines interleukin (IL)-17 and IL-23 are increased in active GPA in conjunction with PR3-specific Thelper-17 cells. Furthermore, IL-15 and cytotoxic CD4-positive T-cells expressing NKG2D are increased in GPA whereas the ligand of these T-cells on the endothelium, that is MIC-A, shows an increased expression as well. Also IL-21 and follicular helper T-cells, promoting the humoral immune response, are increased in GPA. Finally, regulatory T-cells, and possibly also regulatory B-cells, are defective in GPA (data on T-cells are summarised in 13, 14). So, effector T-cells are supposedly involved, in particular in GPA (Fig. 1, ref. 15).

In conclusion, evidence is accumulating that MPO-ANCA is directly involved in the pathogenesis of MPA. In GPA, besides PR3-ANCA, cell-mediated immunity appears to be involved as well in its pathogenesis.

**Table I.** ANCA as present in patients with ANCA-associated vasculitis from a single center.

| Diagnosis | PR3-ANCA | MPO-ANCA | Elastase-ANCA | No ANCA detected | % of patients with ANCA |
|---|---|---|---|---|---|
| GPA (n=364) | 323 | 25 | 4 | 12* | 96 |
| EGPA (n=36) | 0 | 23 | 0 | 13 | 64 |
| MPA (n=85) | 16 | 67 | 1 | 1 | 98 |
| NCGN (n=54) | 4 | 47 | 1 | 2 | 94 |

*10 of 12 were ENT-limited GPA.

## Treatment

Randomised controlled trials from the European Vasculitis Study Group (EUVAS) and the Vasculitis Clinical Research Consortium (VCRC) have set the stage for an evidence-based approach in treating patients with AAV (16, 17). This approach aims to reduce toxicity of treatment, particularly of cyclophosphamide, while maintaining or improving efficacy. Generally, in patients with systemic disease, remission is induced with *i.v.* cyclophosphamide (15 mg/kg every 2 weeks, 3 times, then every 3 weeks) in combination with corticosteroids (3 times 1 gr *i.v.* methylprednisolone followed by 1 mg/kg oral prednisone in a tapering scheme). Plasma exchange is added in case of impending renal insufficiency (serum creatinine >500 μmol/l) and/or life threatening disease. Mycophenolate mofetil (MMF) may be used in case of intolerance to cyclophosphamide. In patients with loco-regional or early systemic disease methotrexate (MTX, 20–25 mg/wk) may be used. For maintenance of remission azathioprine (2 mg/kg/d) is used or MTX (20–25 mg/wk), the latter particularly in case of intolerance to azathioprine. Addition of co-trimoxazole may be considered in case of chronic nasal carriage of staphylococcus aureus and persisting smouldering upper airway disease.

The introduction of rituximab (RTX) has, however, changed the field. The RAVE study showed that 4 infusions of RTX (375 mg/m$^2$/week x 4) were not inferior to oral cyclophosphamide, both in combination with a tapering dose of corticosteroids, for induction of remission in severe AAV. Furthermore, RTX proved superior to cyclophosphamide for patients included with a relapse of their disease. In addition, patients with PR3-ANCA did better than patients with MPO-ANCA (18). For those patients who reached remission in the RAVE trial, no maintenance treatment was given in the RTX-arm whereas patients in the cyclophosphamide arm received azathioprine for maintaining remission. In terms of relapse-free remission, no differences were observed between both arms (19). The RITUXIVAS trial on patients with AAV and severe renal disease also showed that RTX (with 2 infusions of cyclophosphamide) without maintenance treatment was as effective as a full course of *i.v.* cyclophosphamide for induction of remission followed by azathioprine for maintenance (20). Finally, RTX proved superior to azathioprine for maintaining remission in AAV (21). We do need longer follow-up of patients treated with RTX but the data presently available suggest that RTX may replace cyclophosphamide for induction of remission in AAV and replace azathio-

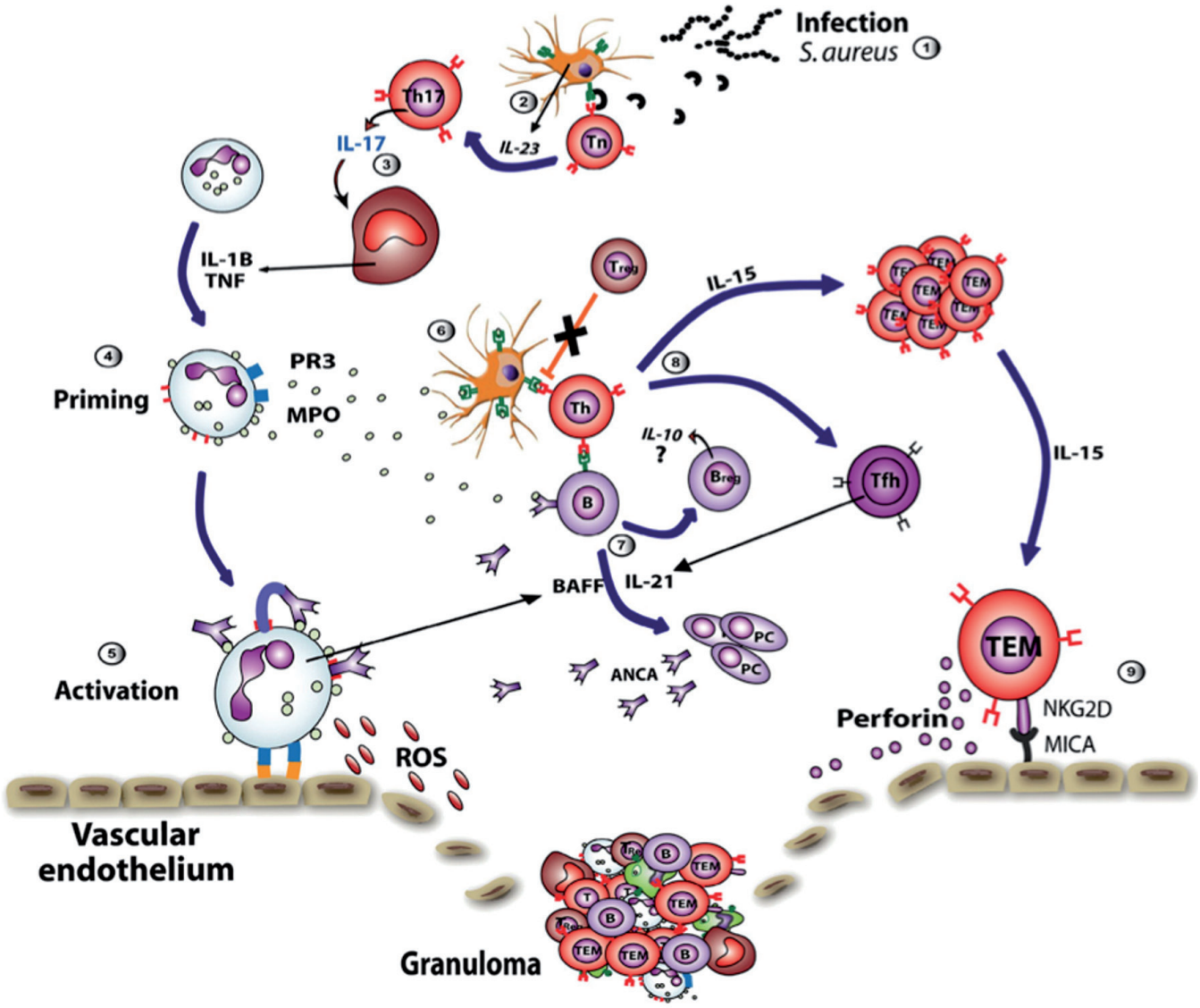


**Fig. 1.** Overview of the innate and adaptive immune system components putatively involved in the development of ANCA-associated vasculitis (1). Peptidoglycan and superantigens from *S. aureus* activate antigen presenting cells (APCs) to (2) produce IL-23, a cytokine necessary to skew and maintain a Th17 cell phenotype (3). IL-17, released from activated Th17 cells, promotes the release of proinflammatory cytokines TNF-α and IL-1β from macrophages (4). These proinflammatory cytokines induce release and translocation of the autoantigens (PR3 and MPO) on the neutrophil cell surface, on one hand, and promote the upregulation of adhesion molecules on both vascular endothelial cells and primed neutrophils, on the other hand (5). Primed neutrophils are being recruited to the site of inflammation and adhere to the vascular endothelium. Next, adherent neutrophils expressing PR3 or MPO on their surface become fully activated by ANCA leading to local production of reactive oxygen species and release of proteolytic enzymes that damage the endothelium (6). Released PR3 or MPO from activated neutrophils, can be internalised and presented by APC to Th cells. Because of the impaired function of Tregs (possible defect in Bregs) in AAV, the autoreactive response can proceed, in which B-cells can differentiate into ANCA-producing plasma cells, whereas Th cells can skew towards Th17 and Tfh cells and expand into TEM cells (7). ANCA can also induce the release of BAFF from neutrophils. BAFF promotes the survival of autoreactive B cells, and together with IL-21 (produced by Tfh cells) synergise in stimulating plasma cell differentiation (8). IL-15 which is overexpressed in AAV patients can promote the expansion of TEM cells bearing the cytotoxic $CD4^{+}NKG2D^{+}$phenotype (9). During active disease, a ligand for NKG2D (that is MICA) becomes upregulated on endothelial cells. TEM cells are attracted to the inflammatory areas and interact with MICA expressed by endothelial cells. This in turn enhances their cytotoxic function leading to endothelial cell killing in a perforin and granzyme dependent way and contributing to granuloma formation. *Reprinted from Reference 15, with permission*

prine for maintaining remission. Currently, RTX is first choice in relapsing patients with PR3-ANCA and/or previously high cyclophosphamide exposure, patients with refractory disease, patients in whom fertility should be protected, and, possibly, patients at risk for co-morbidities.

Finally, could we reduce or even replace the use of corticosteroids for induction of remission? As already mentioned, oral administration of CCX168, a C5a-receptor small molecule antagonist, was shown in an animal model of MPO-ANCA AAV to be protective against disease development and to reduce ongoing disease (22). Very preliminary data from the CLEAR (C5aR inhibitor on Leukocytes Exploratory ANCA-associated Renal vasculitis) trial suggests that oral treatment with this C5a-receptor blocker could be effective in reducing the dose or even eliminating prednisone at all from the

induction regimen for severe (renal) AAV. Data from longer observation periods and from more patients are eagerly awaited.

## Conclusions

As our insight into the pathogenesis of AAV has greatly increased more targeted treatment becomes possible. The RCTs performed by EUVAS and VCRC have set the stage for evidence based treatment of AAV. More recently, RTX has proven effective for induction and maintenance treatment, and now possibilities for reducing steroid dose are on the horizon. Nevertheless, relapsing disease is still a major problem.

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