Anti-Neutrophil Cytoplasm–Associated Glomerulonephritis

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Wegener’s granulomatosis, microscopic polyangiitis, and renal limited vasculitis are associated with circulating anti-neutrophil cytoplasm antibodies and are an important cause of rapidly progressive glomerulonephritis. This review gives an account of recent advances in the understanding of the pathogenesis underlying these conditions and how these may lead to future treatments. Consideration is given to recent clinical trials in the management of anti-neutrophil cytoplasm antibodies (ANCA)-associated vasculitides.

Environmental and Genetic Factors in Pathogenesis of ASV

Because of the low incidence of ASV, it has been difficult to conduct good quality population-based studies to investigate environmental or genetic factors that may contribute to the development or clinical course of ASV. A number of studies have suggested that a variety of environmental factors, such as farming, solvent exposure, earthquake, or a protective effect of smoking, may be linked to development of ASV, although some of these have been contradictory (4–6). The most consistently reported environmental factor is silica exposure, although the mechanism by which this could lead to ASV is unclear (4,7,8). Drug exposure also may precipitate ANCA-associated vasculitis, including propylthiouracil, minocycline, and penicillamine. In those with high titers of anti-MPO ANCA, exposure to these drugs should be considered (9). However, in a prospective study of 248 patients who were treated with penicillamine, sulfasalazine, or minocycline, no patients seroconverted to a positive ANCA (10).

PR3 is produced by neutrophils and can be expressed on the cell surface, where levels may be genetically determined. Membrane expression of PR3 (mPR3) can be measured by flow cytometry, and in most studies, there is a bimodal distribution of mPR3 with both high- and low-mPR3-expressing neutrophils in a given individual (11). Although the total amount of mPR3 can be increased by cytokines, the distribution of high- and low-mPR3-expressing neutrophils has been shown to be stable for an individual over time (12,13). There is evidence that individuals with an increased percentage of high-mPR3-expressing neutrophils are at increased risk for developing WG, whereas patients who already have WG are at increased risk for relapse (14,15). Neutrophils with high mPR3 produce more superoxide in response to PR3-ANCA than neutrophils with low mPR3 (16). Evidence that the proportion of high and low

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mPR3 is genetically determined is derived from twin studies in which a high degree of concordance is seen in monozygotic but not in dizygotic twins (13).

There has been interest in an association between the development of ASV and polymorphisms in the gene encoding α1-antitrypsin (AAT), the major inhibitor of PR3 activity. The allele that most commonly is associated with reduced AAT activity is designated PiZ and is reported to be associated with an increased risk for developing WG (17,18) or either PR3- or MPO-ANCA (19,20). Two studies have reported an increased risk for death for patients who have ASV and carry the PiZ allele (18,20), although a low serum level of AAT was associated with a reduced incidence of renal involvement in one study (20). The mechanism by which carriage of the PiZ allele increases the risk for death for patients with ASV is not clear but, because it is associated with a reduced amount of circulating AAT/PR3 complexes, there may be increased circulating PR3 activity, although this has not yet been demonstrated.

**ANCA Testing and Diagnosis of ASV**

ANCA are detected using indirect immunofluorescence (IIF) of patient serum on ethanol-fixed neutrophils and by ELISA. Several studies have compared the sensitivity and the specificity of both tests used alone or in combination for detection of ANCA. A meta-analysis of 15 studies revealed a positive cytoplasmic ANCA pattern (c-ANCA); IIF had a sensitivity for WG of 34 to 92% and a specificity of 88 to 100%, although the pretest probability of WG was high in most of the groups tested (21). A multicenter study that used standardized IIF and ELISA investigated the specificity and the sensitivity of combined IIF and ELISA in WG, MPA, and RLV. The sensitivity of the combined IIF and ELISA results for newly diagnosed WG, MPA, and RLV are shown in Table 1. The specificity of the combined results was nearly 100% compared with healthy controls (22). However, when clinicians rely on data that are derived from commercial kits, they should be aware that such kits vary widely. One study compared the sensitivity of 11 commercial direct ELISA and found that sensitivity ranged from 26.7 to 86.7% and specificity ranged from 75 to 99.8%, although a low serum level of AAT was associated with a reduced incidence of renal involvement in one study (20). The mechanism by which carriage of the PiZ allele increases the risk for death for patients with ASV is not clear but, because it is associated with a reduced amount of circulating AAT/PR3 complexes, there may be increased circulating PR3 activity, although this has not yet been demonstrated.

**Are ANCA Pathogenic?**

The presence of circulating ANCA is closely associated with ASV and often, although by no means always, reflects disease activity. There are, however, a number of patients who remain ANCA negative despite having a diagnosis of either MPA or WG (25,26). That patients can have active vasculitis with WG or MPA in the absence of ANCA suggests that ANCA themselves may not play a direct role, that ANCA are necessary but not sufficient for disease induction, or that other mediators that can mimic the effects of ANCA exist. Nevertheless, strong evidence of a pathogenic role of ANCA in ASV is provided by animal studies.

A mouse model of MPO-ANCA ASV has been developed in which MPO−/− mice develop murine MPO-ANCA after immunization with murine MPO. Recombinant activating gene 2 deficient (Rag2−/−) and WT6 mice that received murine MPO-ANCA either by passive transfer or by infusion of splenocytes developed renal vasculitis. Glomerular accumulation of neutrophils and macrophages was seen in association with the development of glomerular necrosis and crescents. Mice that underwent neutrophil depletion were protected from the development of renal lesions, supporting the hypothesis that neutrophils play a central role in the pathogenesis of ASV (27).

An animal model of PR3-ANCA ASV has been described. PR3 and neutrophil elastase double-deficient mice were inoculated with murine PR3. These animals developed PR3-ANCA that recognized both murine and human PR3. IgG-containing PR3-ANCA or control IgG was passively transferred to wild-type mice. Intradermal injection of TNF-α in wild-type mice led to development of panniculitis that was significantly worse in mice that received the IgG-containing PR3-ANCA. Passive transfer of antibody to wild-type mice that had received bacterial LPS did not lead to the development of systemic, lung, or renal vasculitis (28). The absence of systemic vasculitis in this model has many possible explanations, not least of which is that the investigators were unable to detect PR3 expressed on the surface of murine neutrophils that were isolated from blood, unlike human neutrophils; PR3 was present on murine neutrophils that were isolated from the peritoneal cavity after TNF-α injection. This suggests that ANCA would be able to interact with neutrophils only in tissue, causing localized inflammation.

Are there other mechanisms that may lead to tissue injury in vasculitis and that do not require the presence of ANCA? Other autoantibodies, for example the anti–endothelial cell antibodies (AECA), discussed next, have been proposed as a pathogenic mechanism, whereas other mechanisms of tissue injury may include the production of matrix metalloproteinases (MMP). A recent study described an increased production of MMP from peripheral blood mononuclear cells and increased plasma levels in patients with active WG compared with healthy control subjects. Although the MMP also were increased in patients who were in remission, this was accompanied by an increase in tissue inhibitors of MMP that was not seen in patients with active disease. The authors of this study suggest that the imbalance of MMP and tissue inhibitors of MMP in patients with active WG may contribute to tissue injury (29).

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**Table 1. Sensitivity of combined positive ELISA and IIF result for new diagnoses of WG, MPA, and RLV**

<table>
<thead>
<tr>
<th></th>
<th>WG (%)</th>
<th>MPA (%)</th>
<th>RLV (%)</th>
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<tbody>
<tr>
<td>p-ANCA with anti-MPO</td>
<td>16</td>
<td>49</td>
<td>46</td>
</tr>
<tr>
<td>c-ANCA with anti-PR3</td>
<td>56 to 58</td>
<td>12 to 16</td>
<td>36</td>
</tr>
</tbody>
</table>

*ANCA, anti-neutrophil cytoplasm antibodies; IIF, indirect immunofluorescence; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; RLV, renal limited vasculitis; WG, Wegener’s granulomatosis.*

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AECAs

For some years, the presence of circulating AECAs has been reported in patients with ASV as well as other inflammatory conditions. These antibodies have been shown to be reactive against constitutively expressed endothelial cell antigens. Incubation of AECAnegative IgG from patients with cultured human umbilical vein endothelial cells induced increased expression of adhesion molecules, cytokines, and chemokines, suggesting that these autoantibodies may promote leukocyte recruitment across endothelium (30). A more recent study using endothelial cells that were cultured from human nasal, kidney, liver sinusoidal, and lung microvascular endothelial cells showed increased binding of AECAs to unstimulated kidney endothelial cells and nasal endothelial cells and cytokine-treated lung microvascular endothelial cells compared with human umbilical vein endothelial cells and liver sinusoidal endothelial cells (31). This raises the possibility that AECAs are specific for the endothelial cells in the target organs of ASV and may promote leukocyte recruitment to these organs via the production of cytokines and increased expression of adhesion molecules, as well as cause endothelial cell injury.

ANCA, Neutrophil, and Macrophage Involvement in ASV

Study of interactions between ANCA and neutrophils has provided insights into potential pathogenic mechanisms of ASV. ANCA activation of neutrophils requires both antigen binding and Fc receptor engagement. Neutrophils from individuals who are deficient in MPO are unable to respond to MPO-ANCA; however, there is no correlation between neutrophil surface expression of MPO and disease activity (32). Patients with ASV show increased transcription of PR3 and MPO-related genes (33,34). Despite ANCA antigen expression on the neutrophil cell surface, the evidence for in vivo binding of PR3-ANCA to neutrophils has been controversial (35,36).

Cross-linking of MPO and PR3 with Fc receptors on the surface of cytokine-primed neutrophils by ANCA in vitro leads to intracellular signal transduction, resulting in the production of superoxide, proinflammatory cytokines, and degranulation (37–39). Interaction of intact ANCA and ANCA F(ab′)2 fragments with neutrophils initiates complementary intracellular signaling cascades, indicating that engagement of both surface antigen and Fc receptors contributes to activation of multiple signaling pathways (40–42) (Figure 1). p21ras, a molecule that is a branch point in multiple signaling cascades, controls a number of cellular processes in the neutrophil, including the respiratory burst. It was shown recently that both intact ANCA IgG and ANCA F(ab′)2 fragments activate only one of the two isoforms of p21ras present in the neutrophil, Kirsten-ras, during initiation of a neutrophil respiratory burst (41). This potentially could permit specific inhibition of the ANCA-activated signaling pathways without complete inhibition of all p21ras functions.

ANCA activation of cytokine-primed neutrophils in vitro leads to accelerated and dysregulated apoptosis that ultimately may lead to neutrophil death by secondary necrosis, thereby promoting inflammation (43). Apoptotic neutrophils also express on the cell surface MPO and PR3 antigens that are capable of binding by ANCA, but these neutrophils cannot undergo cellular activation. The opsonization of apoptotic neutrophils by ANCA induces increased uptake by macrophages and release of proinflammatory cytokines. In vito, this may lead to a failure to resolve inflammation in the normal manner (44,45). In addition, ANCA may have a direct effect on monocytes inducing increased CD11b expression, shedding of CD62L, and the production of superoxide (46).

Role of Cytokines

Circulating neutrophils from patients with ASV are in a more active or “primed” state than those from healthy control subjects with increased expression of activation markers and increased basal superoxide production (45,47). This may be due to circulating proinflammatory cytokines such as TNF-α (48). TNF-α has several effects on neutrophils in vitro that are vital for neutrophils to interact with ANCA and endothelial cells and produce proinflammatory and cytotoxic molecules. TNF-α priming of neutrophils causes the mobilization of PR3 and MPO from granules to the cell surface, as well as transcription of PR3 mRNA and de novo synthesis of PR3 (38,49). Cytokine priming of neutrophils leads to increased expression of β2-integrin adhesion molecules, which are necessary for their interaction with endothelium (50). Cytokine priming is necessary for the ANCA-stimulated production of superoxide by neutrophils. This priming of the respiratory burst involves the phosphorylation and activation of intracellular signaling molecules...
and the mobilization of the components of the NADPH oxidase complex (51–53). Priming also enhances cytokine production (54,55). Cytokines other than TNF-α also have a priming effect on neutrophils, e.g., GM-CSF. Recent work from our institution has demonstrated that IL-18 primes the ANCA-stimulated neutrophil respiratory burst response via the phosphorylation of p38 mitogen-activated protein kinase without increasing the surface expression of PR3 or MPO (56).

The importance of cytokines in this disease is underlined by the animal model using MPO-ANCA described above. Mice that were given LPS and MPO-ANCA developed exaggerated renal injury, associated with a rise in circulating TNF-α. Treatment with anti-TNF-α attenuated the LPS-mediated aggravation of the renal lesion, highlighting the importance of proinflammatory cytokines (57).

**Neutrophil–Endothelial Cell Interactions**

Endothelial injury occurs early in ASV, and there is compelling evidence that ANCA mediates the interactions between cytokine-primed neutrophils and activated endothelium. A large study of 65 renal biopsies from patients with ASV found that neutrophils and macrophages were the predominant leukocytes in glomerular lesions in ASV (58), whereas another study suggested that macrophages were more commonly present than lymphocytes (59). This partly may be because the production of IL-8 and monocyte chemoattractant protein-1 induces recruitment of neutrophils and macrophages across the glomerular capillary endothelium (39,60). Furthermore, ANCA has been shown in vitro to cause changes in the actin cytoskeleton of neutrophils, which could lead to their retention in glomerular capillaries (61).

Interactions among ANCA, neutrophils, and endothelial cells have been studied using in vitro flow models and intravital microscopy. Flow models involve perfusing neutrophils across a monolayer of endothelial cells that have been preactivated with a cytokine to increase the expression of endothelial cell adhesion molecules. After treatment with low concentrations of TNF-α, endothelial cells capture neutrophils from flow and support rolling adhesion via selectin molecules. Perfusion of ANCA over rolling neutrophils induces stationary adhesion of neutrophils to endothelial cells and causes increased transmigration. Both adhesion and transmigration are β2-integrin dependent; transmigration but not adhesion is reduced by the blockade of the chemokine receptor CXCR2 (62–64). ANCA do not alter the level of expression of β2-integrins on neutrophils but do cause a conformational change in CD11b, revealing its activation epitope (63).

Similar effects were seen in an animal model of MPO-ANCA ASV in the WKY rat. ANCA promoted neutrophil attachment to and transmigration through endothelium into mesentery in response to GROα (a rat homologue of IL-8; Figure 2). Rats that were immunized with human MPO or received MPO-ANCA by passive transfer developed evidence of microvascular injury with areas of mesenteric hemorrhage (Figure 3) (65).

The mediators of the endothelial damage that is associated with vasculitis have been widely assumed to comprise both reactive oxygen species and serine protease released from neutrophils. However, a recent study found that serine proteases, rather than reactive oxygen species, were responsible for injury (66), which equates with the ability of PR3 and elastase to induce apoptosis after uptake into endothelial cells (67).

**Role of Lymphocytes in ANCA-Associated Vasculitis**

Circulating T cells in patients with ASV show persistent evidence of activation during active disease and remission, with a Th1-type cytokine profile (68–73). There has been par...
ticular interest in T cells that do not express the co-stimulatory molecule CD28, with expanded populations of both CD4+ CD28− and CD8+ CD28− cells in the peripheral blood of patients with WG; the increase in the CD28− population may correlate with more severe disease manifestations (74). There also is evidence of an increase in circulating peripheral blood memory T cells that express the inducible chemokine receptors CCR3 and CCR5, which may be recruited along chemotactic gradients into sites of inflammation (75,76).

Within tissues, a Th1 cytokine profile has been reported also, particularly in T cells from granulomatous tissue of patients with WG (74). CD4+ CD28− cells, present in granulomas, seem to contribute to production of IFN-γ and TNF-α (77,78). These cells show evidence of an effector-memory phenotype, oligoclony, and replicative senescence, suggesting that they are the product of antigen exposure but not necessarily MPO or PR3 (78,79) (reviewed in [80]).

Given the important role that ANCA probably plays in the pathogenesis of vasculitis, it is surprising that we understand so little about the factors that initiate and control its production. ANCA is largely an IgG class-switched antibody, which suggests that T cell help is involved in its production by B cells and plasma cells.

Both MPO and PR3 are normal human proteins, so regulatory processes that govern the development of T and B cells should prevent the development of autoreactive cells that are capable of causing disease. That ANCA are found in patients with ASV suggests that there has been a failure in these normal regulatory processes.

B cell function is disturbed in patients with active ASV. B cell activation is related to disease activity, and B cells that are capable of producing ANCA seemingly without T cell help have been identified in the circulation of patients with active ASV. The suggested explanation for this is that B cells in these patients have already been maximally stimulated in vivo and therefore may have escaped normal control (72,81).

MPO and PR3 reactive T cells have been demonstrated inconsistently in patients with vasculitis and also are found in normal individuals, calling into question whether MPO and PR3 indeed are the antigens that drive the aberrant T cell populations. Potential other antigens include proteins that are present in *Staphylococcus aureus*, which show homology to human PR3 (82). In one study, peripheral blood mononuclear cells from patients with active WG showed significantly more proliferation to coagulase-positive staphylococci than controls, whereas some T cell lines that were derived from peripheral blood mononuclear cells showed weak cross-reactivity with PR3 (83).

An alternative mechanism for the production of PR3-ANCA was proposed recently. Complementary PR3 (cPR3), a protein that is encoded by the antisense strand of the PR3 encoding gene, may be expressed. Antibodies develop against cPR3 and, in turn, induce anti-idiotypic antibodies that cross-react with PR3 (PR3-ANCA) and lead to the development of ASV (84).

**Lymphocytes and the Development of Glomerular Crescents**

Crescent formation, with little in the way of immune complex deposition, is the hallmark lesion in patients with ANCA-associated vasculitis. A number of animal models of glomerulonephritis have demonstrated a role for T cells in the development of these lesions (see associated article by Tipping and Holdsworth in this issue of JASN85). However these models are dependent on a response against an implanted antigen or hapten in the kidney, which has not been reported in ASV, and the glomerular lesion is notable for its marked T cell component, which is not commonly reported in human ASV (58,59). The relationship between the development of crescents in ASV and the initial focal segmental necrotizing lesions, which frequently contain neutrophils and are pauci-immune in nature, is not clearly understood.

**Treatment of Patients with ASV**

Current treatment strategies for patients with ANCA-associated glomerulonephritis are based on broad immunosuppression using corticosteroids, purine antimetabolites, and alkylating agents. Other approaches are suggested for ASV without renal involvement (86). The introduction of cyclophosphamide and corticosteroids has changed the natural history of the disease from an almost invariably fatal condition to a relapsing and remitting one, with greatly increased survival. However, the use of corticosteroids and cyclophosphamide is associated with a high degree of toxicity and treatment-related morbidity and mortality. The initial management of the disease is induction of remission with aggressive immunosuppression to control inflammation and prevent further organ damage. The second phase of treatment is the maintenance of remission with less intensive immunosuppression to limit the adverse effects of the therapeutic agents while retaining control over the disease. Currently, there is debate as to the intensity of initial immunosuppression and the duration of induction regimens using cyclophosphamide; in addition, it is unclear exactly how long patients should remain on immunosuppression and at what point, if any, immunosuppression can be discontinued safely. As our understanding of the pathogenesis of ASV improves, so should our ability to generate safer, more targeted therapies.

Several recent studies have aimed to optimize the use of corticosteroids and cyclophosphamide in inducing disease remission in ASV, many conducted by the European Vasculitis Study Group (EUVAS; http://www.vasculitis.org). A meta-analysis suggested that pulsed cyclophosphamide may be superior to continuous cyclophosphamide at inducing disease remission, associated with fewer treatment-associated adverse events, although potentially associated with a higher relapse rate (87). Preliminary results of the EUVAS CYCLOPS study, a large, prospective, randomized, controlled, international trial that compared pulsed and continuous cyclophosphamide, has suggested that for non–life- or –organ-threatening disease, pulsed cyclophosphamide is as efficacious as continuous daily oral cyclophosphamide with a lower cumulative dose (88). There was no difference in relapse rates.
More aggressive immunosuppression is required in patients with life- or organ-threatening disease. A number of case series and retrospective studies have suggested benefit of additional plasma exchange for patients with dialysis-dependent renal failure or pulmonary hemorrhage with ASV. A prospective open-label trial of cyclophosphamide and corticosteroids either with or without plasmapheresis for patients with ASV found that plasmapheresis was of additional benefit only to patients who were dialysis dependent at the time of presentation (89). Preliminary results of the EUVAS MEPEX trial have suggested that plasmapheresis was superior to methylprednisolone for recovery of renal function in patients with a creatinine >5.6 mg/100 ml (90).

Cyclophosphamide is extremely effective at inducing and maintaining long-term remission in patients with WG. The regimen used at the National Institutes of Health is cyclophosphamide 2 mg/kg daily until patients have been in full remission for 1 yr. Cyclophosphamide was given in conjunction with a tapering dose of oral corticosteroids (91); although effective in maintaining disease remission, this regimen is associated with adverse effects, including bone marrow suppression, gonadal suppression, and hemorrhagic cystitis. The incidence of bladder cancer in patients with WG that were treated with cyclophosphamide, was reported as 5% at 10 yr, representing a 31-fold increase compared with the general population (92). This has led to studies that have examined whether WG or MPA can be treated safely with shorter courses of cyclophosphamide to reduce the risk of treatment-related morbidity. The EUVAS CYCAZAREM trial, which compared 12 mo of cyclophosphamide treatment with early cessation of cyclophosphamide and substitution of azathioprine as maintenance therapy, showed equivalence in both groups for patient survival, relapse rate, disease activity, and renal function; there was a nonsignificant reduction of adverse events in those who were randomly assigned to receive azathioprine after disease remission. This supports an early change to less toxic azathioprine when the patient has entered remission (93). A caveat to this is that in a study of disease-free survival in patients with PR3-ANCA ASV, patients who had a positive PR3-ANCA test at the moment of switching from cyclophosphamide to azathioprine had a relative risk for relapse of 2.6 compared with those who were ANCA negative. Patients who were ANCA negative at switching to azathioprine had the same risk for relapse as patients who received cyclophosphamide only (94). Several EUVAS trials are ongoing, investigating the most effective drugs and duration of treatment for maintenance of disease remission.

Newer therapies that are based on improved understanding of the pathogenesis of vasculitis also have been reported. The proinflammatory role of TNF-α in ASV led to several reports and pilot studies that used anti–TNF-α therapies. An open-label pilot study of 20 patients who had WG and were given etanercept (TNF receptor fusion protein) in addition to their existing immunosuppression suggested that the drug was safe and probably effective in controlling WG disease activity (95). An open-label pilot study of 32 patients who had WG and MPA and received infliximab (anti-TNF mAb) in addition to standard therapy for new, relapsed, or resistant disease suggested that infliximab may be effective in controlling ASV, although there were a number of relapses during or soon after infliximab therapy, as well as a number of significant infectious episodes (96).

As a result of these pilot studies, a randomized, controlled trial of etanercept compared with placebo, added to standard immunosuppression, for the induction and maintenance of remission of WG was conducted in 180 patients. The trial failed to show any benefit in adding etanercept to standard immunosuppression (97). The relapse rates in both groups were high, and >55% of patients in both groups had at least one severe or life-threatening adverse event. Six patients died, although none as a result of active WG. Significantly more patients developed solid-organ cancers in the etanercept group than in the control group. All of the patients who developed cancer had received cyclophosphamide during the trial, and several had received previous prolonged courses of cyclophosphamide, which may have increased their risk for developing cancer. One possible criticism of the design of this clinical trial is that, although the patients were stratified by disease severity before randomization, the trial was not powered to detect any differences in outcome for subgroups of patients with more or less severe disease, limited or generalized disease, or newly diagnosed or relapsed disease. The etanercept group was less likely to achieve sustained remission, but this group contained significantly more patients who had pre-existing disease and may have been less likely to achieve a sustained remission, although the disease duration was not significantly different between the two groups.

Differences in the efficacy of the various anti-TNF therapies have been seen in the treatment of other granulomatous diseases, such as Crohn’s disease and sarcoidosis, in which infliximab but not etanercept is of benefit (98,99). Etanercept predominantly binds to soluble TNF-α, whereas infliximab also binds to membrane-bound TNF-α, and this may lead to infliximab-specific effects such as lysis or apoptosis of membrane TNF-α-expressing cells (100,101). Although etanercept has not been shown to be of benefit in WG, large-scale trials using anti–TNF-α antibodies would be justified as its different biologic actions may prove to be therapeutically useful in either WG or MPA.

Other recently reported new treatments that show promise for ASV include the anti–B cell therapy rituximab (anti-CD20). Several case and small series reports of its safe, effective use in resistant or difficult-to-manage ASV have now been reported (99,102), although two recent, prospective, open-label studies have reported both the successful and the unsuccessful use of rituximab in patients with ASV (103,104). Despite the fact that CD20 is not expressed on plasma cells, the use of rituximab is associated with a reduction in ANCA titer as well as B cell depletion, without evidence of a generalized immunoparesis (105). It has been suggested that the reduction in ANCA that is seen after administration of rituximab is due to the concurrent administration of corticosteroids (106) and that rituximab may owe any therapeutic efficacy to interference with other B cell functions; however, there also are reports of the reduction in ANCA titers in the absence of steroids (103) or with an unchanged corticosteroid dose (105). If the ability of rituximab to reduce ANCA titers is confirmed, then a better understanding of the way that this antibody seems to
Table 2. Treatment recommendations tailored to EUVAS disease severity classification

<table>
<thead>
<tr>
<th>Disease Classification</th>
<th>Definition</th>
<th>Treatment</th>
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<tr>
<td>Early systemic vasculitis</td>
<td>Serum creatinine &lt;120 μmol/L, constitutional symptoms, no threat to any vital organ function, positive or negative ANCA</td>
<td>Methotrexate and steroids for disease remission induction</td>
</tr>
<tr>
<td>Generalized vasculitis</td>
<td>Serum creatinine &lt;500 μmol/L, dysfunction of any vital organ, positive ANCA</td>
<td>Cyclophosphamide and steroids for remission induction (87), then change to azathioprine and steroids (93)</td>
</tr>
<tr>
<td>Severe renal vasculitis</td>
<td>Serum creatinine &gt;500 μmol/L, constitutional symptoms, positive ANCA</td>
<td>Plasma exchange, cyclophosphamide, and steroids for remission induction (90), then change to azathioprine and steroids for maintenance</td>
</tr>
<tr>
<td>Refractory vasculitis</td>
<td>Any serum creatinine, constitutional symptoms, threatened function of any vital organ, positive or negative ANCA</td>
<td>No randomized, controlled trial evidence</td>
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*See reference (114) for details.*

induce selective deletion of ANCA-producing cells would greatly improve our understanding of the mechanisms that control ANCA production. Either way, the role of rituximab both as a potential therapeutic intervention for patients with ASV and as a tool to help define the role that B cells play in ASV merit further investigation. Currently, the Immune Tolerance Network is recruiting patients to a prospective, randomized, controlled, double-blind trial of rituximab in patients with ASV (http://www.immunetolerance.org/RAVE/).

Anti–T cell therapies using anti-thymocyte globulin or anti-CD4 or anti-CD52 antibodies have been reported in small series. Remission has been achieved using these agents in patients with resistant vasculitis, albeit with high relapse rates and a high rate of infectious complications (107–109).

Treatment should be tailored to disease severity. The current evidence-based treatments are shown in Table 2 according to EUVAS disease severity classification. Other possible future therapeutic strategies might include new anticytokine therapies such as IL-18–binding protein to reduce neutrophil priming and endothelial activation, intervention in the neutrophil-endothelium adhesion cascade, or the neutrophil intracellular signaling pathways that are activated by ANCA.

**Prognostic Markers and Outcome**

Disease mortality has significantly improved to approximately 80% survival at 5 yr with introduction of current therapeutic regimens. Poor prognostic markers that are consistently associated with ANCA-associated vasculitis include older age and more severe renal impairment (110–112). Renal biopsy gives further information regarding outcome. Chronic lesions, particularly interstitial fibrosis, are associated with poor renal outcome and correlate with renal function at 18 mo (86,113). It is of interest that there are no specific differences between WG and MPA in renal pathology; however, patients with MPA are likely to present with more chronic lesions.

Treatment-related morbidity is common; 25% of patients experience adverse effects of treatment within the first year, with infection being the most common cause of early death. Identification of those who are at greatest risk for morbidity is important when weighing benefits of potent immunosuppressive treatments. However, we have no good markers to identify those who will respond uniformly poorly to treatment.

**Conclusion**

Laboratory and clinical research has improved our understanding of the complex interplay of the components of the immune system and the roles that they play in the pathogenesis of ASV. As our understanding of the pathogenesis has improved, particularly of the role of ANCA, this has improved our understanding of why empirically derived broad immunosuppression has been effective in controlling this autoimmune disease. Our current understanding has led to the development of newer therapies for ASV, targeted at specific immune system components, that will potentially provide better disease control with less treatment-related morbidity and mortality.

**Acknowledgments**

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**References**

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