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.

Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and renal limited vasculitis (RLV) are inflammatory autoimmune conditions that commonly are associated with circulating autoantibodies. These antibodies, known as anti-neutrophil cytoplasm antibodies (ANCA), are directed against proteinase 3 (PR3) or myeloperoxidase (MPO) that is present in the primary granules of neutrophils and monocytes. MPA and WG are systemic diseases that often affect several organ systems; are associated with nonspecific inflammatory features such as fever, malaise, weight loss, and anemia; and are seen most commonly in the elderly, with a peak age of onset between 65 and 74 yr (1). These conditions are commonly grouped together as the ANCA-associated systemic vasculitides (ASV); therefore, they show common histologic features in the kidney and ANCA are thought to contribute to pathogenesis.

Detection of ANCA has aided diagnosis, as there is a high sensitivity and specificity of these antibodies for disease. As well as being associated with MPA and WG, less commonly ANCA may be present during infection or associated with drug-induced vasculitis when their significance is unclear, as they are often directed against other neutrophil antigens.

The renal features of ASV include oliguria, microscopic hematuria, and proteinuria. This may be associated with an impaired GFR, although renal function as measured by serum creatinine may be normal. The gold standard test for the presence of glomerulonephritis remains renal biopsy. The histopathologic features have been reviewed in two recent studies (2,3), but the central feature is a pauci-immune focal segmental necrotizing glomerulonephritis that may become crescentic with the accumulation of macrophages and epithelial cells in Bowman's space. This review concentrates on recent developments in the diagnosis, pathogenesis, and management of glomerulonephritis associated with WG, MPA, and RLV.

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 α-1-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 from 13.3 to 73.3% for MPO- and PR3-ANCA, respectively, compared with IIF. Sensitivity can be increased by changing the cutoff point but leads to a reduction in specificity for diagnosis of ASV (23). Results of ANCA testing also vary between centers, as shown by one multicenter international study in which the same sera were tested in different sites (24).

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

	WG (%)	MPA (%)	RLV (%)
p-ANCA with anti-MPO	16	49	46
c-ANCA with anti-PR3	56 to 58	12 to 16	36

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

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 WTB6 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).

AECA

For some years, the presence of circulating AECA 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 AECA-containing 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 AECA 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 AECA 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
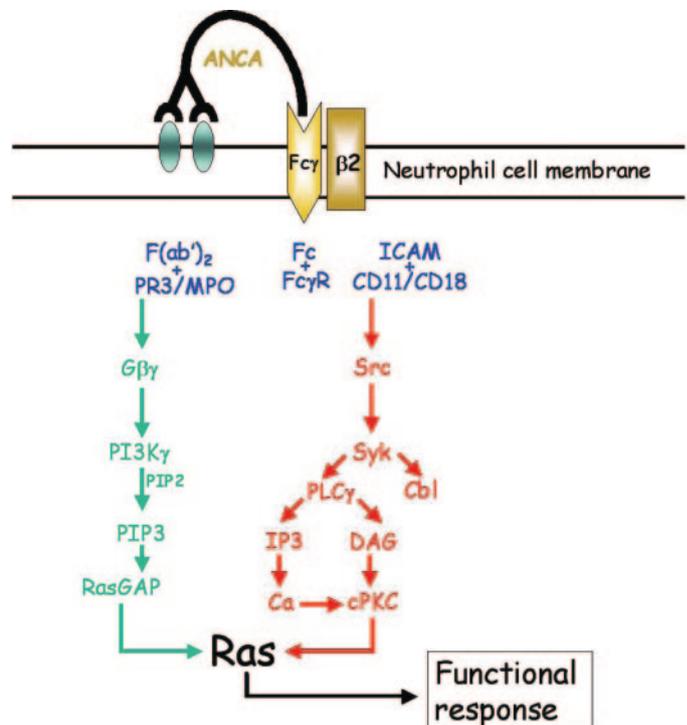

Figure 1. Signaling pathways in neutrophil activated by anti-neutrophil cytoplasm antibodies (ANCA) F(ab')$_2$ fragment and whole antibody.

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 vivo*, 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 $\beta2$-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 $\beta2$-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 neu-
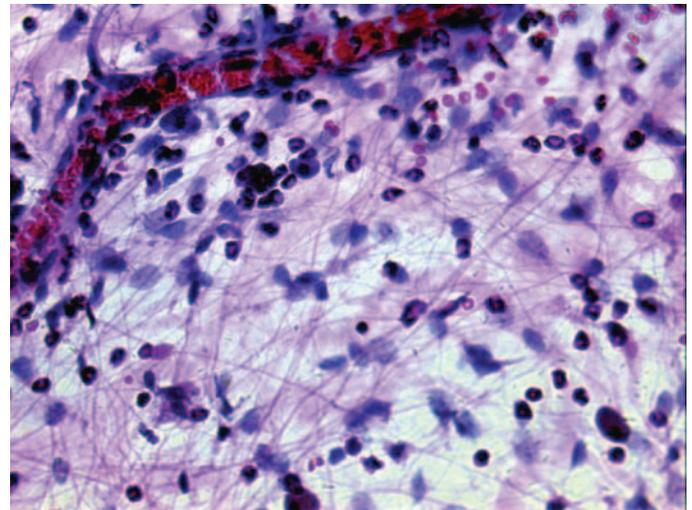

Figure 2. Neutrophil firm adhesion and transmigration in the mesentery of a WKY rat after induction of ANCA by immunization with myeloperoxidase (MPO). A topical inflammatory stimulus was provided by mesenteric superfusion with CXCL-1 (3×10^{-9} M). This process may be quantified in real time using intravital microscopy. Magnification, $\times40$, hematoxylin and eosin.
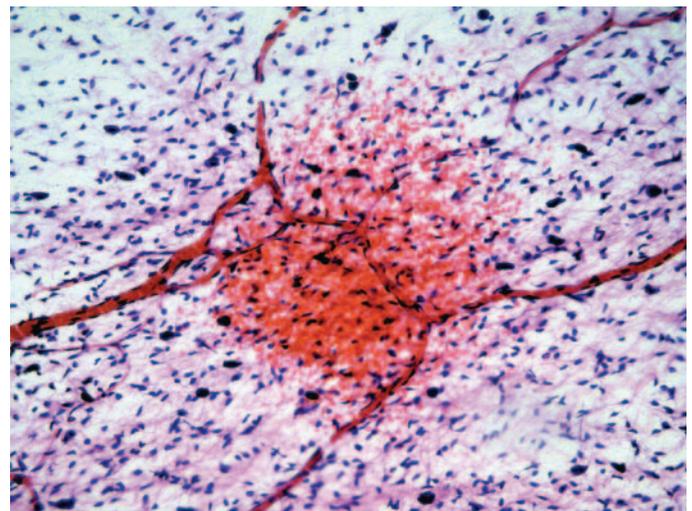

Figure 3. Mesenteric microvascular hemorrhage in a WKY rat after infusion of anti-MPO antibodies and superfusion with CXCL-1 (3×10^{-9} M). Magnification, $\times20$, hematoxylin and eosin.

trophils. 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, oligoclonality, 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-idiotype 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 *JASN*85). 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 glomerulonephritides 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 is 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 ef-

fective 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[a]

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

[a]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

References

1. Watts R, Lane S, Bentham G, Scott D: Epidemiology of systemic vasculitis. A ten-year study in the United Kingdom. *Arthritis Rheum* 43: 414–419, 2000
2. Hauer H, Bajema I, van Houwelingen H, Ferrario F, Noel

L, Waldherr R, Jayne D, Rasmussen N, Bruijn J, Hagen E, European Vasculitis Study Group, (EUVAS): Renal histology in ANCA-associated vasculitis: Differences between diagnostic and serologic subgroups. *Kidney Int* 61: 80–89, 2002

3. Seo P, Stone JH: The antineutrophil cytoplasmic antibody-associated vasculitides. *Am J Med* 117: 39–50, 2004

4. Lane SE, Watts RA, Bentham G, Innes NJ, Scott DGI: Are environmental factors important in primary systemic vasculitis? A case-control study. *Arthritis Rheum* 48: 814–823, 2003

5. Haubitz M, Woywodt A, de Groot K, Haller H, Goebel U: Smoking habits in patients diagnosed with ANCA associated small vessel vasculitis. *Ann Rheum Dis* 64: 1500–1502, 2005

6. Yashiro M, Muso E, Itoh-Ihara T, Oyama A, Hashimoto K, Kawamura T, Ono T, Sasayama S: Significantly high regional morbidity of MPO-ANCA-related angitis and/or nephritis with respiratory tract involvement after the 1995 great earthquake in Kobe (Japan). *Am J Kidney Dis* 35: 889–895, 2000

7. Hogan SL, Satterly KK, Dooley MA, Nachman PH, Jennette JC, Falk RJ: Silica exposure in anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and lupus nephritis. *J Am Soc Nephrol* 12: 134–142, 2001

8. Gregorini G, Ferioli A, Donato F, Tira P, Morassi L, Tardanico R, Lancini L, Maiorca R: Association between silica exposure and necrotizing crescentic glomerulonephritis with p-ANCA and anti-MPO antibodies: A hospital-based case-control study. *Adv Exp Med Biol* 336: 435–440, 1993

9. Choi HK, Merkel PA, Walker AM, Niles JL: Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: Prevalence among patients with high titers of antimyeloperoxidase antibodies. *Arthritis Rheum* 43: 405–413, 2000

10. Choi HK, Slot MC, Pan G, Weissbach CA, Niles JL, Merkel PA: Evaluation of antineutrophil cytoplasmic antibody seroconversion induced by minocycline, sulfasalazine, or penicillamine. *Arthritis Rheum* 43: 2488–2492, 2000

11. Muller-Kobold ACM, Kallenburg CGM, Tervaert JWC: Leukocyte membrane expression of proteinase 3 correlates with disease activity in patients with Wegener's granulomatosis. *Br J Rheumatol* 37: 901–907, 1998

12. Halbwachs-Mecarelli L, Bessou G, Lesavre P, Lopez S, Witko-Sarsat V: Bimodal distribution of proteinase 3 (PR3) surface expression reflects a constitutive heterogeneity in the polymorphonuclear neutrophil pool. *FEBS Lett* 374: 29–33, 1995

13. Schreiber A, Busjahn A, Luft FC, Kettritz R: Membrane expression of proteinase 3 is genetically determined. *J Am Soc Nephrol* 14: 68–75, 2003

14. Rarok AA, Stegeman CA, Limburg PC, Kallenberg CGM: Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. *J Am Soc Nephrol* 13: 2232–2238, 2002

15. Witko-Sarsat V, Lesavre P, Lopez S, Bessou G, Hieblot C, Prum B, Noel LH, Guillevin L, Ravaud P, Sermet-Gaudelus I, Timsit J, Grunfeld JP, Halbwachs-Mecarelli L: A large subset of neutrophils expressing membrane proteinase 3 is a risk factor for vasculitis and rheumatoid arthritis. *J Am Soc Nephrol* 10: 1224–1233, 1999

16. Schreiber A, Luft FC, Kettritz R: Membrane proteinase 3

17. expression and ANCA-induced neutrophil activation. *Kidney Int* 65: 2172–2183, 2004

17. Baslund B, Szpirt W, Eriksson S, Elzouki AN, Wiik A, Wieslander J, Petersen J: Complexes between proteinase 3, alpha 1-antitrypsin and proteinase 3 anti-neutrophil cytoplasm autoantibodies: A comparison between alpha 1-antitrypsin PiZ allele carriers and non-carriers with Wegener's granulomatosis. *Eur J Clin Invest* 26: 786–792, 1996

18. Elzouki A, Segelmark M, Wieslander J, Eriksson S: Strong link between the alpha 1-antitrypsin PiZ allele and Wegener's granulomatosis. *J Intern Med* 236: 543–548, 1994

19. Esnault VL, Testa A, Audrain M, Roge C, Hamidou M, Barrier JH, Sesboue R, Martin JP, Lesavre P: Alpha 1-antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis. *Kidney Int* 43: 1329–1332, 1993

20. Callea F, Gregorini G, Sinico A, Gonzales G, Bossolasco M, Salvidio G, Radice A, Tira P, Candiano G, Rossi G, Petti A, Ravera G, Ghiggeri G, Gusmano R: Alpha1-antitrypsin (AAT) deficiency and ANCA-positive systemic vasculitis: Genetic and clinical implications. *Eur J Clin Invest* 27: 696–702, 1997

21. Rao JK, Weinberger M, Oddone EZ, Allen NB, Landsman P, Feussner JR: The role of antineutrophil cytoplasmic antibody (c-ANCA) testing in the diagnosis of Wegener granulomatosis. A literature review and meta-analysis. *Ann Intern Med* 123: 925–932, 1995

22. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, Lesavre P, Ludemann J, Rasmussen N, Sinico RA, Wiik A, van der Woude FJ: Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int* 53: 743–753, 1998

23. Holle JU, Hellmich B, Backes M, Gross WL, Csernok E: Variations in performance characteristics of commercial enzyme immunoassay kits for detection of antineutrophil cytoplasmic antibodies: What is the optimal cut off? *Ann Rheum Dis* 64: 1773–1779, 2005

24. Csernok E, Holle J, Hellmich B, Willem J, Tervaert C, Kallenberg CGM, Limburg PC, Niles J, Pan G, Specks U, Westman K, Wieslander J, De Groot K, Gross WL: Evaluation of capture ELISA for detection of antineutrophil cytoplasmic antibodies directed against proteinase 3 in Wegener's granulomatosis: First results from a multicentre study. *Rheumatology* 43: 174–180, 2004

25. Lane SE, Watts RA, Shepstone L, Scott DGI: Primary systemic vasculitis: Clinical features and mortality. *QJM* 98: 97–111, 2005

26. Westman KWA, Selga D, Isberg P-E, Bladstrom A, Olsson H: High proteinase 3-anti-neutrophil cytoplasmic antibody (ANCA) level measured by the capture enzyme-linked immunosorbent assay method is associated with decreased patient survival in ANCA-associated vasculitis with renal involvement. *J Am Soc Nephrol* 14: 2926–2933, 2003

27. Xiao H, Heeringa P, Liu Z, Huugen D, Hu P, Maeda N, Falk RJ, Jennette JC: The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am J Pathol* 167: 39–45, 2005

28. Pfister H, Ollert M, Frohlich LF, Quintanilla-Martinez L, Colby TV, Specks U, Jenne DE: Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood* 104: 1411–1418, 2004

29. Bjerkeli V, Halvorsen B, Damas JK, Nordoy I, Yndestad A, Aukrust P, Froland SS: Expression of matrix metalloproteinases in patients with Wegener's granulomatosis. *Ann Rheum Dis* 63: 1659–1663, 2004

30. Carvalho D, Savage CO, Isenberg D, Pearson JD: IgG anti-endothelial cell autoantibodies from patients with systemic lupus erythematosus or systemic vasculitis stimulate the release of two endothelial cell-derived mediators, which enhance adhesion molecule expression and leukocyte adhesion in an autocrine manner. *Arthritis Rheum* 42: 631–640, 1999

31. Holmen C, Christensson M, Pettersson E, Bratt J, Stjarne P, Karrar A, Sumitran-Holgersson S: Wegener's granulomatosis is associated with organ-specific antiendothelial cell antibodies. *Kidney Int* 66: 1049–1060, 2004

32. Reumaux D, de Boer M, Meijer AB, Duthilleul P, Roos D: Expression of myeloperoxidase (MPO) by neutrophils is necessary for their activation by anti-neutrophil cytoplasm autoantibodies (ANCA) against MPO. *J Leukoc Biol* 73: 841–849, 2003

33. Yang JJ, Pendergraft WF, Alcorta DA, Nachman PH, Hogan SL, Thomas RP, Sullivan P, Jennette JC, Falk RJ, Preston GA: Circumvention of normal constraints on granule protein gene expression in peripheral blood neutrophils and monocytes of patients with antineutrophil cytoplasmic autoantibody-associated glomerulonephritis. *J Am Soc Nephrol* 15: 2103–2114, 2004

34. Reynolds WF, Stegeman CA, Cohen Tervaert JW: −463 G/A myeloperoxidase promoter polymorphism is associated with clinical manifestations and the course of disease in MPO-ANCA-associated vasculitis. *Clin Immunol* 103: 154–160, 2002

35. Van Rossum A, van der Geld Y, Limburg P, Kallenberg C: Human anti-neutrophil cytoplasm autoantibodies to proteinase 3 (PR3-ANCA) bind to neutrophils. *Kidney Int* 68: 537–541, 2005

36. Abdel-Salam B, Iking-Konert C, Schneider M, Andrassy K, Hansch G: Autoantibodies to neutrophil cytoplasmic antigens (ANCA) do not bind to polymorphonuclear neutrophils in blood. *Kidney Int* 66: 1009–1017, 2004

37. Kettritz R, Jennette J, Falk R: Crosslinking of ANCA-antigens stimulates superoxide release by human neutrophils. *J Am Soc Nephrol* 8: 386–394, 1997

38. Falk R, Terrell R, Charles L, Jennette J: Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A* 87: 4115–4119, 1990

39. Cockwell P, Brooks C, Adu D, Savage C: Interleukin-8: A pathogenetic role in antineutrophil cytoplasmic autoantibody-associated glomerulonephritis. *Kidney Int* 55: 1125–1127, 1999

40. Kettritz R, Choi M, Butt W, Rane M, Rolle S, Luft FC, Klein JB: Phosphatidylinositol 3-kinase controls antineutrophil cytoplasmic antibodies–induced respiratory burst in human neutrophils. *J Am Soc Nephrol* 13: 1740–1749, 2002

41. Williams JM, Savage COS: Characterization of the regulation and functional consequences of p21ras activation in neutrophils by antineutrophil cytoplasm antibodies. *J Am Soc Nephrol* 16: 90–96, 2005

42. Ben-Smith A, Dove SK, Martin A, Wakelam MJO, Savage COS: Antineutrophil cytoplasm autoantibodies from patients with systemic vasculitis activate neutrophils through distinct signaling cascades: comparison with conventional Fcgamma receptor ligation. *Blood* 98: 1448–1455, 2001

43. Harper L, Ren Y, Savill J, Adu D, Savage COS: Antineutrophil cytoplasmic antibodies induce reactive oxygen-dependent dysregulation of primed neutrophil apoptosis and clearance by macrophages. *Am J Pathol* 157: 211–220, 2000

44. Moosig F, Csernok E, Kumanovics G, Gross W: Opsonization of apoptotic neutrophils by anti-neutrophil cytoplasmic antibodies (ANCA) leads to enhanced uptake by macrophages and increased release of tumour necrosis factor-alpha (TNF-alpha). *Clin Exp Immunol* 122: 499–503, 2000

45. Harper L, Cockwell P, Adu D, Savage CO: Neutrophil priming and apoptosis in anti-neutrophil cytoplasmic autoantibody-associated vasculitis. *Kidney Int* 59: 1729–1738, 2001

46. Wikman A, Fagergren A, Forslid J, Jacobson S, Johansson S, Lundahl J: Antineutrophil cytoplasmic antibodies induce decreased CD62L expression and enhanced metabolic activity in monocytes. *Scand J Immunol* 57: 179–184, 2003

47. Muller-Kobold A, Mesander G, Stegeman C, Kallenberg C, Tervaert J: Are circulating neutrophils intravascularly activated in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides? *Clin Exp Immunol* 114: 491–499, 1998

48. Arimura Y, Minoshima S, Kamiya Y, Tanaka U, Nakabayashi K, Kitamoto K, Nagasawa T, Sasaki T, Suzuki K: Serum myeloperoxidase and serum cytokines in anti-myeloperoxidase antibody-associated glomerulonephritis. *Clin Nephrol* 40: 256–264, 1993

49. Zhou Z, Richard C, Menard H: De novo synthesis of proteinase 3 by cytokine primed circulating human polymorphonuclear neutrophils and mononuclear cells. *J Rheumatol* 27: 2406–2411, 2000

50. Condliffe A, Chilvers E, Haslett C, Dransfield I: Priming differentially regulates neutrophil adhesion molecule expression/function. *Immunology* 89: 105–111, 1996

51. Dewas C, Dang PM-C, Gougerot-Pocidalo M-A, El-Benna J: TNF-alpha induces phosphorylation of p47phox in human neutrophils: Partial phosphorylation of p47phox is a common event of priming of human neutrophils by TNF-alpha and granulocyte-macrophage colony-stimulating factor. *J Immunol* 171: 4392–4398, 2003

52. Kettritz R, Schreiber A, Luft FC, Haller H: Role of mitogen-activated protein kinases in activation of human neutrophils by antineutrophil cytoplasmic antibodies. *J Am Soc Nephrol* 12: 37–46, 2001

53. McLeish K, Knall C, Ward R, Gerwins P, Coxon P, Klein J, Johnson G: Activation of mitogen-activated protein kinase cascades during priming of human neutrophils by TNF-alpha and GM-CSF. *J Leukoc Biol* 64: 537–545, 1998

54. Yuo A, Kitagawa S, Kasahara T, Matsushima K, Saito M, Takaku F: Stimulation and priming of human neutrophils by interleukin-8: Cooperation with tumor necrosis factor and colony-stimulating factors. *Blood* 78: 2708–2714, 1991

55. Jablonska E, Kiluk M, Markiewicz W, Jablonski J: Priming effects of GM-CSF, IFN-gamma and TNF-alpha on human neutrophil inflammatory cytokine production. *Melanoma Res* 12: 123–128, 2002

56. Hewins P, Morgan M, Holden N, Neil D, Williams J, Savage C, Harper L: IL-18 is up-regulated in the kidney and primes neutrophil responsiveness in ANCA-associated vasculitis. *Kidney Int* 69: 605–615, 2006

57. Huugen D, Xiao H, van Esch A, Falk RJ, Peutz-Kootstra CJ,

Buurman WA, Tervaert JWC, Jennette JC, Heeringa P: Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: Role of tumor necrosis factor-alpha. *Am J Pathol* 167: 47–58, 2005

58. Weidner S, Carl M, Riess R, Rupprecht HD: Histologic analysis of renal leukocyte infiltration in antineutrophil cytoplasmic antibody-associated vasculitis: Importance of monocyte and neutrophil infiltration in tissue damage. *Arthritis Rheum* 50: 3651–3657, 2004

59. Cunningham MA, Huang XR, Dowling JP, Tipping PG, Holdsworth SR: Prominence of cell-mediated immunity effectors in "pauci-immune" glomerulonephritis. *J Am Soc Nephrol* 10: 499–506, 1999

60. Cockwell P, Howie A, Adu D, Savage C: In situ analysis of C-C chemokine mRNA in human glomerulonephritis. *Kidney Int* 54: 827–836, 1998

61. Tse W, Nash G, Hewins P, Savage C, Adu D: ANCA-induced neutrophil F-actin polymerization: Implications for microvascular inflammation. *Kidney Int* 67: 130–139, 2005

62. Radford DJ, Luu NT, Hewins P, Nash GB, Savage COS: Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum* 44: 2851–2861, 2001

63. Calderwood JW, Williams JM, Morgan MD, Nash GB, Savage COS: ANCA induces beta2 integrin and CXC chemokine-dependent neutrophil-endothelial cell interactions that mimic those of highly cytokine-activated endothelium. *J Leukoc Biol* 77: 33–43, 2005

64. Smith C, Marlin S, Rothlein R, Toman C, Anderson D: Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 83: 2008–2017, 1989

65. Little MA, Smyth CL, Yadav R, Ambrose L, Cook HT, Nourshargh S, Pusey CD: Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood* 106: 2050–2058, 2005

66. Lu X, Garfield A, Rainger GE, Savage COS, Nash GB: Serine proteases but not superoxide released from ANCA-stimulated neutrophils mediate endothelial cell damage. *Arthritis Rheum* 2006, in press

67. Yang JJ, Preston GA, Pendergraft WF, Segelmark M, Heeringa P, Hogan SL, Jennette JC, Falk RJ: Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am J Pathol* 158: 581–592, 2001

68. Gutfleisch J, Baumert E, Wolff-Vorbeck G, Schlesier M, Strutz H, Peter H: Increased expression of CD25 and adhesion molecules on peripheral blood lymphocytes of patients with Wegener's granulomatosis (WG) and ANCA positive vasculitides. *Adv Exp Med Biol* 336: 397–404, 1993

69. Schlesier M, Kaspar T, Gutfleisch J, Wolff-Vorbeck G, Peter H: Activated CD4+ and CD8+ T-cell subsets in Wegener's granulomatosis. *Rheumatol Int* 14: 213–219, 1995

70. Ikeda M, Watanabe Y, Kitahara S, Inouye T: Distinctive increases in HLA-DR+ and CD8+57+ lymphocyte subsets in Wegener's granulomatosis. *Int Arch Allergy Immunol* 102: 205–208, 1993

71. Ludviksson BR: Active Wegener's granulomatosis is associated with HLA-DR+CD4+ T-cells exhibiting an unbalanced Th1-type T-cell cytokine pattern: Reversal with IL-10. *J Immunol* 160: 3602–3609, 1998

72. Popa ER, Stegeman CA, Bos NA, Kallenberg CG, Tervaert JW: Differential B- and T-cell activation in Wegener's granulomatosis. *J Allergy Clin Immunol* 103: 885–894, 1999

73. Csernok E, Trabandt A, Muller A, Wang G, Moosig F, Paulsen J, Schnabel A, Gross W: Cytokine profiles in Wegener's granulomatosis: Predominance of type 1 (Th1) in the granulomatous inflammation. *Arthritis Rheum* 42: 742–750, 1999

74. Moosig F, Csernok E, Wang G, Gross W: Costimulatory molecules in Wegener's granulomatosis (WG): Lack of expression of CD28 and preferential up-regulation of its ligands B7-1 (CD80) and B7-2 (CD86) on T cells. *Clin Exp Immunol* 114: 113–118, 1998

75. Lamprecht P, Erdmann A, Mueller A, Csernok E, Reinhold-Keller E, Holl-Ulrich K, Feller AC, Bruehl H, Gross WL: Heterogeneity of CD4+ and CD8+ memory T cells in localized and generalized Wegener's granulomatosis. *Arthritis Res Ther* 5: R25–R31, 2003

76. Lamprecht P, Bruhl H, Erdmann A, Holl-Ulrich K, Csernok E, Seitzer U, Mack M, Feller AC, Reinhold-Keller E, Gross WL, Muller A: Differences in CCR5 expression on peripheral blood CD4+CD28− T-cells and in granulomatous lesions between localized and generalized Wegener's granulomatosis. *Clin Immunol* 108: 1–7, 2003

77. Lamprecht P, Moosig F, Csernok E, Seitzer U, Schnabel A, Mueller A, Gross W: CD28 negative T-cells are enriched in granulomatous lesions of the respiratory tract in Wegener's granulomatosis. *Thorax* 56: 751–757, 2001

78. Komocsi A, Lamprecht P, Csernok E, Mueller A, Holl-Ulrich K, Seitzer U, Moosig F, Schnabel A, Gross W: Peripheral blood and granuloma CD4+CD28− T-cells are a major source of interferon gamma and tumour necrosis factor alpha in Wegener's granulomatosis. *Am J Pathol* 160: 1717–1724, 2002

79. Vogt S, Iking-Konert C, Hug F, Andrassy K, Hansch GM: Shortening of telomeres: Evidence for replicative senescence of T cells derived from patients with Wegener's granulomatosis. *Kidney Int* 63: 2144–2151, 2003

80. Lamprecht P: Off balance: T-cells in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides. *Clin Exp Immunol* 141: 201–210, 2005

81. Clayton A, Savage C: Production of antineutrophil cytoplasm antibodies derived from circulating B cells in patients with systemic vasculitis. *Clin Exp Immunol* 132: 174–179, 2003

82. Lawyer C, Henkle J, Bakir H: Nasal carriage of staphylococcal infection in Wegener granulomatosis. *Ann Intern Med* 121: 74–75, 1994

83. Mayet W, Marker-Hermann E, Schlaak J, Meyer Zum Buschenfelde K: Staphylococcal superantigens and T cell expansions in Wegener's granulomatosis. *Clin Exp Immunol* 49: 585–594, 1999

84. Pendergraft W 3rd, Preston G, Shah R, Tropsha A, Carter CJ, Jennette J, Falk R: Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med* 10: 72–79, 2004

85. Tipping PG, Holdsworth SR: T cells in crescentic glomerulonephritis. *J Am Soc Nephrol* 17: 1253–1263, 2006

86. Jayne D: Update on the European Vasculitis Study Group trials. *Curr Opin Rheumatol* 13: 48–55, 2001

87. de Groot K, Adu D, Savage COS: The value of pulse cyclophosphamide in ANCA-associated vasculitis: Meta-analysis and critical review. *Nephrol Dial Transplant* 16: 2018–2027, 2001

88. de Groot K, Jayne D, Tesar V, Savage C; EUVAS investigators: Randomised controlled trial of daily oral versus pulse cyclophosphamide for induction of remission in ANCA-associated systemic vasculitis [Abstract]. *Kidney Blood Press Res* 28: 103, 2005

89. Pusey C, Rees A, Evans D, Peters D, Lockwood C: Plasma exchange in focal necrotizing glomerulonephritis without anti-GBM antibodies. *Kidney Int* 40: 757–763, 1991

90. Gaskin G, Jayne D, Group EVS. Adjunctive plasma exchange is superior to methylprednisolone in acute renal failure due to ANCA-associated glomerulonephritis [Abstract]. *J Am Soc Nephrol* 13: 2A, 2002

91. Fauci A, Haynes B, Katz P, Wolff S: Wegener's granulomatosis: Prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 98: 76–85, 1983

92. Talar-Williams C, Hijazi YM, Walther MM, Linehan WM, Hallahan CW, Lubensky I, Kerr GS, Hoffman GS, Fauci AS, Sneller MC: Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med* 124: 477–484, 1996

93. Jayne D, Rasmussen N, Andrassy K, Bacon P, Tervaert JWC, Dadoniene J, Ekstrand A, Gaskin G, Gregorini G, de Groot K, Gross W, Hagen EC, Mirapeix E, Pettersson E, Siegert C, Sinico A, Tesar V, Westman K, Pusey C; the European Vasculitis Study Group: A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 349: 36–44, 2003

94. Slot MC, Tervaert JW, Boomsma MM, Stegeman CA: Positive classic antineutrophil cytoplasmic antibody (C-ANCA) titer at switch to azathioprine therapy associated with relapse in proteinase 3-related vasculitis. *Arthritis Rheum* 51: 269–273, 2004

95. Stone JH: Etanercept combined with conventional treatment in Wegener's granulomatosis. *Arthritis Rheum* 44: 1149–1154, 2001

96. Booth A, Harper L, Hammad T, Bacon P, Griffith M, Levy J, Savage C, Pusey C, Jayne D: Prospective study of TNFalpha blockade with infliximab in anti-neutrophil cytoplasmic antibody-associated systemic vasculitis. *J Am Soc Nephrol* 15: 717–721, 2004

97. The Wegener's Granulomatosis Etanercept Trial (WGET) Research Group: Etanercept plus standard therapy for Wegener's granulomatosis. *N Engl J Med* 352: 351–361, 2005

98. Sandborn W, Hanauer S, Katz S, Safdi M, Wolf D, Baerg R, Tremaine W, Johnson T, Diehl N, Zinsmeister A: Etanercept for active Crohn's disease: A randomized, double-blind, placebo-controlled trial. *Gastroenterology* 121: 1088–1094, 2001

99. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR: Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. *Chest* 124: 177–185, 2003

100. Scallon B, Cai A, Solowski N, Rosenberg A, Song X, Shealy D, Wagner C: Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther* 301: 418–426, 2002

101. Haraoui B: Differentiating the efficacy of the tumor necrosis factor inhibitors. *Semin Arthritis Rheum* 34: 7–11, 2005

102. Keogh KA, Wylam ME, Stone JH, Specks U: Induction of remission by B lymphocyte depletion in eleven patients with refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 52: 262–268, 2005

103. Keogh KA, Ytterberg SR, Fervenza FC, Carlson KA, Schroeder DR, Specks U: Rituximab for refractory Wegener's granulomatosis: Report of a prospective, open-label pilot trial. *Am J Respir Crit Care Med* 173: 180–187, 2006

104. Aries PM, Hellmich B, Both M, Nolle B, Voswinkel J, Holl-Ulrich K, Lamprecht P, Gross WL: Lack of efficacy of rituximab in Wegener's granulomatosis with refractory granulomatous manifestations. *Ann Rheum Dis* Nov 3, 2005 [epub ahead of print]

105. Ferraro AJ, Day CJ, Drayson MT, Savage CO: Effective therapeutic use of rituximab in refractory Wegener's granulomatosis. *Nephrol Dial Transplant* 20: 622–625, 2005

106. Sneller MC: Rituximab and Wegener's granulomatosis: Are B cells a target in vasculitis treatment? *Arthritis Rheum* 52: 1–5, 2005

107. Hagen E, de Keizer R, Andrassy K, van Boven W, Bruijn J, van Es L, van der Woude F: Compassionate treatment of Wegener's granulomatosis with rabbit anti-thymocyte globulin. *Clin Nephrol* 43: 351–359, 1995

108. Schmitt W, Hagen E, Neumann I, Nowack R, Flores-Suarez L, van der Woude F; European Vasculitis Study Group: Treatment of refractory Wegener's granulomatosis with antithymocyte globulin (ATG): An open study in 15 patients. *Kidney Int* 65: 1440–1448, 2004

109. Lockwood C, Thiru S, Stewart S, Hale G, Isaacs J, Wraight P, Elliott J, Waldmann H: Treatment of refractory Wegener's granulomatosis with humanized monoclonal antibodies. *QJM* 89: 903–912, 1996

110. Booth AD, Almond MK, Burns A, Ellis P, Gaskin G, Neild GH, Plaisance M, Pusey CD, Jayne DR: Outcome of ANCA-associated renal vasculitis: A 5-year retrospective study. *Am J Kidney Dis* 41: 776–784, 2003

111. Hogan SL, Nachman PH, Wilkman AS, Jennette JC, Falk RJ: Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol* 7: 23–32, 1996

112. Hauer HA, Bajema IM, Van Houwelingen HC, Ferrario F, Noel L-H, Waldherr R, Jayne DRW, Rasmussen N, Bruijn JA, Hagen EC: Determinants of outcome in ANCA-associated glomerulonephritis: A prospective clinico-histopathological analysis of 96 patients. *Kidney Int* 62: 1732–1742, 2002

113. Vergunst CE, van Gurp E, Hagen EC, van Houwelingen HC, Hauer HA, Noel LH, Waldherr R, Ferrario F, van der Woude FJ, Bruijn JA, Bajema IM: An index for renal outcome in ANCA-associated glomerulonephritis. *Am J Kidney Dis* 41: 532–538, 2003

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