ANCA-associated vasculitides: advances in pathophysiology and treatment

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ABSTRACT

Substantial progress has been made over the last two decades in our understanding of the immunopathogenesis of antineutrophil cytoplasmic antibodies (ANCA) associated vasculitides. Compelling evidence from *in vitro* studies and experimental models in conjunction with clinical trials has confirmed that ANCA directly contribute to the evolution and progression of the disease process. Continuous development in our understanding of the mechanisms that drive the disease may ultimately allow us to tailor the multitude of novel therapies, which are rapidly becoming available, to the requirements of individual patients.

In this review we endeavour to provide a brief overview of the recent advances in ANCA-associated vasculitides and outline basic principles for diagnosis and treatment of these complex multisystem diseases.

KEYWORDS

ANCA, vasculitis, autoimmune disease, Wegeners granulomatosis, microscopic polyangiitis, Churg Strauss vasculitis

INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) associated vasculitides principally comprises three overlapping but distinct disease entities: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS). ANCA-associated systemic vasculitis (AAV) has an incidence of 20 per million and a prevalence of 144 per million in the UK.[1] In vasculitis, the blood vessels are the primary site of autoimmune inflammation, and the pathological consequence is destruction of the vessel wall, seen histologically as fibrinoid necrosis. The disease may be limited to a single organ or vascular bed, but commonly affects multiple organ systems. AAV has substantial morbidity and mortality, often presenting with aggressive renal failure or pulmonary haemorrhage, and requiring therapy with toxic immunosuppression.[2] Left untreated, AAV has a universally poor prognosis with mortality approaching 100% within five years.[3] The introduction of treatment regimens based on cyclophosphamide and glucocorticoids has transformed AAV from a rapidly fatal disease, to one of chronic morbidity and reduced survival often preceded by end-stage renal disease.

ANCA were originally discovered by chance in the early 1980s by Davies *et al.* in serum samples from patients with necrotising glomerulonephritis.[4] They became serological hallmarks of the disease after Van der Woude showed that they mainly occur in WG.[5] They are directed against constituents of granules of neutrophils and lysosomes of monocytes. Indirect immunofluorescence detects two distinct patterns perinuclear (pANCA) and cytoplasmic (cANCA). Myeloperoxidase (MPO) is the primary antigenic target of pANCAs, and proteinase 3 (PR3) the major autoantigen associated with cANCAs, as determined by antigen-specific ELISA. Positive immunofluorescence in conjunction with a positive ELISA is highly specific for AAV, i.e. >95% of double-positive patients with suspected nephritis reveal vasculitis on renal biopsy. PR3-ANCAs are mainly detected in WG, whereas MPO-ANCAs are predominantly found in MPA and CSS. The availability of ANCA testing has profoundly reduced the diagnostic delay in these conditions, but at the same time increased the median age at diagnosis and the incidence of vasculitis. This suggests that there was a considerable underdiagnosis of vasculitis, particularly in the elderly, in the past.

The pathogenesis of AAV has not been fully elucidated, but a number of genetic and environmental factors have been implicated. There are striking geographic differences

in the incidence of the different AAVs (i.e. WG, the most common form of AAV in northern and central Europe, is virtually nonexistent in Japan and rare in China).[6] The most strongly linked environmental factors are silica exposure, infections particularly with *Staphylococcus aureus* and drugs such as propylthiouracil.[6]

DIAGNOSIS

Early diagnosis of AAV is crucial to prevent irreversible organ damage and to allow initiation of appropriate therapy. Heterogeneity in severity and organ distribution are the biggest stumbling blocks to making a speedy diagnosis. AAV should be on the list of differential diagnoses in any patient presenting with an inflammatory condition.

Patients will typically have fluctuating constitutional symptoms, which may vary in intensity and include malaise, tiredness, mild fevers and nonspecific aches and pains. The prodromal phase of AAV usually lasts months and may often be misinterpreted as viral infection, postviral syndrome or malignancy.

The kidney is the most commonly affected organ in AAV. Renal involvement is initially asymptomatic until renal failure occurs. The first sign of rapidly progressive glomerulonephritis is frequently the detection of blood and protein on urinary dipstick testing, which are always present in renal vasculitis. The presence of red cell casts in the urine sediment, combined with a rapidly rising serum creatinine, is strongly significant for a crescentic glomerulonephritis, as seen in AAV.[2,7]

Patients with AAV frequently present with symptoms suggestive of upper respiratory tract infections including sinusitis, deafness or hoarseness. More aggressive nasal inflammation, nasal septal defects, necrosis and collapse of the bridge, and severe nasal crusting or bleeding are more commonly associated with WG.[8] Lower respiratory tract involvement includes cough, exertional dyspnoea and haemoptysis. Chest X-rays may reveal the characteristic cavitating pulmonary lesions or raise suspicion of pulmonary haemorrhage. Maturity onset asthma in conjunction with a raised eosinophil count is a classic feature of CSS.

Skin lesions are common and can range from a painless purpuric rash to necrotic ulceration.

The involvement or isolated disease of the orbit is a feature of AAV, and usually presents with episcleritis and scleritis; this can lead to loss of vision, especially in cases of retinal vasculitis, retinal venous infarction or retro-orbital granulomas.

Gastrointestinal involvement in AAV is rare, but is more commonly seen in other vasculitic conditions such Henoch-Schoenlein purpura and polyarteriitis nodosa.

Mononeuritis multiplex of peripheral nerves with an axonal pattern is common in AAV, particularly CSS,[9] but direct involvement of the brain is rarely seen at presentation.

Cardiac disease is not common in AAV, but valvular infarction can occur and eosinophilic cardiomyopathy occurs in a significant number of patients with severe CSS. The combination of prodromal symptoms, with one or many characteristic organ manifestations, in conjunction with a positive ANCA titre, leads to a probable diagnosis of AAV. Obtaining a confirmatory biopsy is highly recommended prior to committing patients to prolonged immunosuppression and will help to optimise treatment decisions. ANCA levels may be negative in early or limited disease, and occasional patients with biopsy-confirmed disease remain persistently ANCA negative.

TREATMENT

Early diagnosis must be associated with rapid therapy induction to prevent irreversible organ damage, such as renal scarring, visual impairment and peripheral nerve weakness. The therapeutic goal is to control disease activity and prevent relapse, while minimising risks of acute immunosuppression and late complications, such as cardiovascular disease, osteoporosis and malignancy.

The initial treatment phase is aimed at achieving remission and standard induction therapy with cyclophosphamide and glucocorticoids can induce remission in up to 90% of patients.[10] Cyclophosphamide can be administered as continuous or intravenous/oral therapy, with equal rates of remission induction. However, pulsed strategies have a lower cumulative exposure and fewer cases of leucopenia, but a higher relapse rate (*tables 1* and *2*).[11] In those with more severe disease, plasma exchange improves renal survival,[12] and is widely used in patients with pulmonary haemorrhage, but without clear evidence to support this particular indication. Methotrexate (MTX) can be substituted for cyclophosphamide for remission induction in less severe early systemic disease, with fewer side effects but a higher relapse rate.[13] One small randomised trial of renal vasculitis shows similar remission induction rates for mycophenolate mofetil (MMF) and cyclophosphamide,[14] and the efficacy of MMF is currently being studied in a large multicentre trial (MYCYC). Reducing treatment toxicity is a key emphasis of ongoing trials through substitution of cyclophosphamide with potentially less toxic agents, such as MMF, or by reducing cumulative steroid exposure. A recent study assessing outcome and adverse events of 524 newly diagnosed patients with AAV prospectively recruited to four European trials shows that overall one-year mortality was 11.1%. Importantly, 59% of those deaths were secondary to adverse events and only 14% to disease activity.[15] This clearly shows that in the first year the greatest risk to patients is therapy-associated adverse events.

Table 1. *European Vasculitis Study Group (EUVAS) Trials*

Trial	Number of Patients	Description	Outcome
CYCAZEREM	WG 95 MPA 60	Cyclophosphamide *vs* AZT for maintenance therapy	Replacement of cyclophosphamide with AZT does not increase relapse rate
NORAM	WG 94 MPA 6	MTX *vs* cyclophosphamide for induction of remission	MTX achieved comparable rates of remission but higher relapse rates
CYCLOPS	WG 61 MPA 99	Pulsed *vs* continuous oral cyclophosphamide for remission induction	Equal remission induction, higher relapse rates in pulsed regimens
MEPEX	WG 42 MPA 95	Plasma exchange *vs* high-dose methylpred-nisolone as adjunctive therapy in patients with severe renal impairment	Increased rate of renal recovery in plasma exchange group
RITUXVAS	Total 44	Rituximab *vs* cyclophosphamide for disease induction in renal AAV	Completed recruitment but results not yet published
MYCYC	72 recruited to date	MMF *vs* cyclophosphamide for induction of remission	Recruiting – target 140 patients
PEXIVAS	Target 500	Double cross over to examine steroid dose and plasma exchange for induction of remission	Protocol agreed, ethical approval, not yet recruiting

WG = Wegener's granulomatosis; MPA = microscopic polyangiitis; AZT = azathioprine; MTX = methotrexate; AAV = ANCA-associated vasculitides; MMF = mycophenolate mofetil.

Table 2. *Drugs commonly used to treat AAV*

Drug	Dose	Mechanism of action
Cyclophosphamide	Oral 2 mg/kg/day Pulse 15 mg/kg every 2 to 3 weeks Patients over 65 years of age not more than 100 mg/day (oral)	Alkylating agent – breakdown products covalently bind DNA leading to mutations and apoptosis
Azathioprine	Oral 2 mg/kg/day	Purine antimetabolite interferes with de novo purine synthesis and impairs cell proliferation
Methotrexate	Starting dose 10 mg/kg/once weekly Dose can be titrated up to maximum 25 mg/kg	Inhibition of dihydrofolate reductase thereby disrupting DNA synthesis and cell division
Prednisolone	Standard dose for remission induction 1 mg/kg/day Dose reduction during induction to ~12,5 mg/day at 3 months	Inhibits cytokine, chemokine synthesis and release. Reduces localisation of inflammatory cells to inflamed areas
Mycophenolate	Standard dose 1 or 1.5 g twice daily. Gradually increase dose to reduce gastrointestinal side effects	Inhibits de novo guanine synthesis. Lymphocytes lack salvage pathway resulting in impaired proliferation, antibody production and adhesion

The value of B-cell depletion by anti-CD20 antibodies has been tested in two randomised prospective controlled trials (RITUXVAS and RAVE, the latter was also double blind) *(table 1)*. Importantly, anti-CD20 was used with reduced doses of cyclophosphamide: only two pulses were given in RITUXVAS and none in RAVE. Although these studies have been completed, they are not yet published; however, preliminary reports suggest efficacy that is at least equivalent to cyclophosphamide. An advantage of rituximab is its lesser immunosuppressive properties.

Following remission, usually after three months, cyclophosphamide is replaced by azathioprine (AZT) or MTX and continued for at least 18 months to reduce relapse risk *(table 1)*.[16] Relapse is more common in WG than in MPA, and this has been associated with nasal carriage of *Staphylococcus aureus*.[17] Patients who remain ANCA positive after induction therapy have a fourfold higher risk of relapse than those who become ANCA negative.[18] Generally speaking, patients who receive more potent induction therapy tend to have a lower rate of relapse when treatment is reduced or withdrawn.[11,13] AZT and MTX are associated with intolerance in a small number of cases, and fail to maintain remission in approximately 30% of patients. A significant proportion of patients relapsing on AZT can be rescued by switching to MTX, but MMF is increasingly used as a second-line agent in patients who relapse during maintenance therapy.

Careful monitoring of patients in remission is important as it contributes to early relapse detection and therapy adjustment, limits relapse severity and prevents further irreversible organ damage. Another important consideration of long-term follow-up is to carefully monitor disease and therapy-associated organ damage. Patients with AAV have a twofold increased risk of developing malignancies in comparison with the general population. The risk increase varies according to the affected organ and is highest for bladder cancer, nonmelanoma skin cancer and lymphoma.[19] Measures to reduce the risk of cardiovascular disease should be integral to the management of systemic vasculitis. A

recent study by Morgan *et al.* shows that patients with AAV have a greater risk of cardiovascular disease, in particular patients with pre-diagnosis cardiovascular disease and those with markedly impaired renal function.[20] Osteoporosis risk is markedly increased, especially in those patients who are exposed to a high cumulative steroid dose and prophylaxis is recommended to reduce fracture risk.[21]

A significant number of patients develop refractory disease.[22] Anti-T-cell therapies using antithymocyte globulin or anti-CD52 antibodies have been tested in small studies with some efficacy.[23,24] However, high relapse rates have occurred after withdrawal of therapy, as well as high rates of infectious complications. 15-Deoxyspergualine, a novel immunosuppressant whose mode of action remains unclear but is suggested to include suppression of NF-κB, has shown efficacy with respect to induction of remission in open-label studies of refractory patients with AAV but, as with anti-T-cell therapies, relapses occur rapidly after discontinuation of the drug.[25,26] Rituximab has been used for refractory disease and in a cross-sectional study of 64 patients, full remission was achieved in 75% and partial remission in 23%. Relapses occurred in 58% of patients who had achieved a full remission; 38 of these patients were retreated, with 84% achieving a further full remission.[27] Finally, the initial promise of anti-TNF therapies was not fulfilled in a randomised, controlled trial of etanercept compared with placebo, added to standard therapy for induction and maintenance of remission in WG.[28] Many patients had pre-existing disease and had received prior immunosuppressive therapies, including cyclophosphamide, which may have contributed to the enhanced number of solid organ tumours that occurred in the etanercept group.

LESSONS FROM ANIMAL MODELS/ ANCA PATHOGENICITY

A pathogenetic role of ANCA has been postulated since their close association with small-vessel vasculitis was discovered. Clinically, ANCA titres are frequently related to disease activity or relapse.[29] Therefore, a rising ANCA in a patient in clinical remission should raise suspicion of relapse and increase vigilance and follow-up frequency. *In vitro* experiments show that ANCA induce neutrophil activation by engagement of their target antigens MPO and PR3.[30] Adhesion studies under flow conditions, in which neutrophils are perfused through glass microslides coated with platelets or endothelial cells, show that ANCA play an important role in adhesion and migration. Activation of endothelial cells with low concentrations of TNFα followed by infusion of ANCA IgG resulted

in stabilised adhesion and a tenfold increase in the number of transmigrating neutrophils.[31,32] Adhesion and migration require activation of neutrophil β2 integrins and involve the chemokine receptor CXCR2.[33]

A number of experimental models provide evidence that MPO-ANCA can induce crescentic glomerulonephritis, pulmonary capillaritis and systemic vasculitis. Immunisation of MPO-knockout mice with murine MPO induced MPO-ANCA, and when these were injected, immunodeficient or wild-type mice developed pauci-immune focal necrotising glomerulonephritis.[34] A similar approach did generate PR3-ANCA, but passive transfer of these did not induce vasculitis,[35] but significantly aggravated the local inflammatory response induced by subcutaneous TNF-α administration, thus providing evidence to support PR3-mediated tissue damage *in vivo*.

Little *et al.* have developed a rat model of focal necrotising crescentic glomerulonephritis and pulmonary capillaritis induced through immunisation with purified human MPO. This model has the advantage that it specified the amount of MPO required to induce disease, and more importantly, all animals developed renal and pulmonary damage with reduced variability in severity between animals.[36,37] In the same study, Little *et al.* explored the effects of MPO-ANCA on the induction of leucocyte-endothelium interactions using intravital microscopy of mesenteric venules.[36] Localised administration of the chemokine CXCL-1 (a rat homologue of interleukin-8) to the mesenterium of both immunised and naive rats, pretreated with purified IgG from sera of MPO-immunised rats, led to increased leucocyte adherence, transmigration and focal haemorrhage at chemokine application sites. This work confirms the direct pathogenic effect of MPO-ANCA, and suggests that ANCA pathogenicity is at least in part mediated through promotion of neutrophil adhesion to endothelium *in vivo*.

It has been assumed that complement activation is not involved in the pathogenesis of AAV because of the paucity of immunoglobulin and complement deposits in affected blood vessels and the absence of hypocomplementaemia. Recent evidence, however, points to an important role of complement activation in AAV; *in vitro* activation of human neutrophils by MPO-ANCA or PR3-ANCA leads to complement activation including activation of C3a.[38] *In vivo* complement depletion with cobra venom factor prevented the development of vasculitis following injection of MPO IgG or transfer of anti-MPO splenocytes. Furthermore, a common complement pathway inhibiting C5 antibody prevented or ameliorated MPO IgG-mediated glomerulonephritis when given before or after disease induction, respectively.[39] Studies using mice deficient in specific

complement pathways show that MPO IgG-mediated glomerulonephritis is dependent on the alternative complement pathway.[38] Immunofluorescence microscopy shows deposition of the complement component C3c in glomerular capillaries or mesangium in 33% of patients with AAV and this was associated with elevated proteinuria and more severe renal injury.[40] Overall, these studies support a crucial role for alternative pathway complement activation in AAV and suggest that complement inhibition may be a target for future therapies.

Recently, Kain *et al.*[41] were able to induce pauci-immune glomerulonephritis by immunising rats with rabbit immunoglobulin specific to human lysosomal-associated membrane protein-2 (LAMP-2), which cross-reacts with rat LAMP-2. All rats developed severe renal injury; 22% of glomeruli exhibited focal capillary necrosis after 24 hours, and 21% of glomeruli developed crescents within 48 hours. Anti-human LAMP-2 interestingly shares 100% homology and cross-reacts with the bacterial adhesin FimH, which also induces antibodies to human and rat LAMP-2, as well as causing pauci-immune glomerulonephritis when injected into rats, indicating that fimbriated Gram-negative bacteria are involved in the pathogenesis of AAV by triggering autoimmunity. In the study by Kain *et al.*, anti-LAMP-2 was detectable in almost all patients with focal necrotising glomerulonephritis, which represents a much higher prevalence than that of ANCA directed against PR3 or MPO. This raises the question of whether these antibodies are specific for AAV or more generally involved in vasculitic lesions.

SUMMARY

It has become increasingly clear over the last two decades that ANCA IgG is pathogenic in AAV. This is supported by evidence from *in vitro* and *in vivo* animal studies, as well as clinical observations from patients with AAV. At the same time, ANCA testing has contributed enormously to more rapid diagnosis of new cases, and early recognition of relapse in patients with AAV. Conventional treatments, such as cyclophosphamide and glucocorticoids, have remained the mainstay of therapy in generalised disease, but some progress has been made in minimising treatment dose. Novel therapies aimed at selected cell populations or blocking specific pathogenic pathways offer hope for more selectively treating this heterogeneous group of patients, while avoiding nonspecific immunosuppression and its adverse effects. However, this will not only require controlled trials to evaluate these novel therapies, but also a concerted effort to further enhance our understanding of the mechanisms behind ANCA pathogenesis.

REFERENCES

1. Watts RA, Scott DG, Jayne DR, et al. Renal vasculitis in Japan and the UK – are there differences in epidemiology and clinical phenotype? Nephrol Dial Transplant. 2008;23:3928-31.

2. Erwig LP, Rees AJ. Rapidly progressive glomerulonephritis. J Nephrol. 1999;12:111-9.

3. Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. Am J Kidney Dis. 2003;41:776-84.

4. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? Br Med J. 1982;285:606.

5. Van der Woude FJ, Rasmussen N, Lobatto S, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet. 1985;1:425-9.

6. Chen M, Kallenberg CEG. The environment, geoepidemiology and ANCA-associated vasculitides. Autoimmun Rev. 2009. November. Epub.

7. Morgan MD, Harper L, Williams J, Savage C. Anti-neutrophil cytoplasm-associated glomerulonephritis. J Am Soc Nephrol. 2006;17:1224-34.

8. Masi AT, Hunder GG, Lie JT, et al. The American College of Rheumatology criteria for the classification of Churg Strauss syndrome. Arthritis Rheum. 1990;33:1094-1100.

9. Nishino H, Rubino FA, DeRemee RA, et al. Neurological involvement in Wegener's granulomatosis: an analysis of 324 consecutive patients at the Mayo Clinic. Ann Neurol. 1993;33:4-9.

10. de Groot K, Adu D, Savage CO; EUVAS (European Vasculitis Study Group). The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. Nephrol Dial Transplant. 2001;10:2018-27.

11. de Groot K, Harper L, Jayne DR, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. Ann Intern Med. 2009;150:670-80.

12. Jayne DR, Gaskin G, Rasmussen N, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol. 2007;18:2180-8.

13. De Groot K, Rasmussen N, Bacon PA, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2005;52(8):2461-9.

14. Koukoulaki M, Jayne DR. Mycophenolate mofetil in anti-neutrophil cytoplasm antibodies-associated systemic vasculitis. Nephron Clin Pract. 2006;102:100-7.

15. Little MA, Nightingale P, Verburgh CA, et al. Ann Rheum Dis. 2009;1. Epub ahead of print.

16. Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. N Engl J Med. 2003;349(1):36-44.

17. Popa ER, Stegeman CA, Kallenberg CG, Tervaert JW. Staphylococcus aureus and Wegener's granulomatosis. Arthritis Res. 2002;4(2):77-9.

18. Mukhtyar C, Flossmann O, Hellmich B, et al. Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force. Ann Rheum Dis. 2008;67(7):1004-10.

19. Reinhold-Keller E, Beuge N, Latza U, et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. Arthritis Rheum. 2000;43:1021-32.

20. Morgan MD, Turnbull J, Selamet U, et al. Increased incidence of cardiovascular events in patients with antineutrophil cytoplasmic antibody-associated vasculitides: a matched-pair cohort study. Arthritis Rheum. 2009;60:3493-500.

21. Boomsma MM, Stegeman CA, Kramer AB, Karsijns M, Piers DA, Tervaert JW. Prevalence of reduced bone mineral density in patients with anti-neutrophil cytoplasmic antibody associated vasculitis and the role of immunosuppressive therapy: a cross-sectional study. Osteoporos Int. 2002;13(1):74-82.

22. Schmitt WH, Hagen EC, Neumann I, Nowack R, Flores-Suárez LF, van der Woude FJ; for the European Vasculitis Study Group. Treatment of refractory Wegener's granulomatosis with antithymocyte globulin (ATG): an open study in 15 patients. Kidney Int. 2004;65(4):1440-8.

23. Klein I, Vervoort G, Steenbergen E, Wetzels J. Progressive renal disease despite immunosuppressive therapy in a patient with Wegener's granulomatosis. Neth J Med. 2008;66(3):125-7.

24. Lockwood CM, Thiru S, Stewart S, et al. Treatment of refractory Wegener's granulomatosis with humanized monoclonal antibodies. QJM. 1996;89(12):903-12.

25. Birck R, Warnatz K, Lorenz HM. 15-Deoxyspergualin in patients with refractory ANCA-associated systemic vasculitis: a six-month open-label trial to evaluate safety and efficacy. J Am Soc Nephrol. 2003;14(2):440-7.

26. Schmitt WH, Birck R, Heinzel PA, et al. Prolonged treatment of refractory Wegener's granulomatosis with 15-deoxyspergualin: an open study in seven patients. Nephrol Dial Transplant. 2005;20(6):1083-92.

27. Jones RB, Ferraro AJ, Chaudhry AN, et al. A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum. 2009;60(7):2156-68.

28. Wegener's Granulomatosis Etanercept Trial (WGET) Research Group. Etanercept plus standard therapy for Wegener's granulomatosis. N Engl J Med. 2005;352(4):351-61.

29. Tervaert JW, van der Woude FJ, Fauci AS, et al. Association between active Wegener's granulomatosis and anticytoplasmic antibodies. Arch Intern Med. 1989;149(11):2461-5.

30. Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. Proc Natl Acad Sci USA. 1990;87(11):4115-9.

31. Nolan SL, Kalia N, Nash GB, Kamel D, Heeringa P, Savage CO. Mechanisms of ANCA-mediated leukocyte-endothelial cell interactions in vivo. J Am Soc Nephrol. 2008;19(5):973-84.

32. Little MA, Savage CO. The role of the endothelium in systemic small vessel vasculitis. Clin Exp Rheumatol. 2008;26:S135-40.

33. Calderwood JW, Williams JM, Morgan MD, Nash GB, Savage CO. ANCA induces beta2 integrin and CXC chemokine-dependent neutrophil-endothelial cell interactions that mimic those of highly cytokine-activated endothelium. J Leukoc Biol. 2005;77(1):33-43.

34. Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest. 2002;110(7):955-63.

35. Pfister H, Ollert M, Fröhlich LF, et al. Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. Blood. 2004;104(5):1411-8.

36. Little MA, Smyth CL, Yadav R, et al. Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. Blood. 2005;106(6):2050-8.

37. Little MA, Smyth L, Salama AD, et al. Experimental autoimmune vasculitis: an animal model of anti-neutrophil cytoplasmic autoantibody-associated systemic vasculitis. Am J Pathol. 2009;174(4):1212-20.

38. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. Am J Pathol. 2007;170(1):52-64.

39. Huugen D, van Esch A, Xiao H, et al. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. Kidney Int. 2007;71(7):646-54.

40. Xing GQ, Chen M, Liu G, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. J Clin Immunol. 2009;29(3):282-91.

41. Kain R, Exner M, Brandes R, et al. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. Nat Med. 2008;14(10):1088-96.

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