Pathophysiological importance of anti-neutrophil antibodies in vasculitis

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Abstract

**Purpose of Review**—In this review we endeavour to provide a brief overview of the recent advances in understanding of how anti-neutrophil cytoplasmic antibodies (ANCA) contribute to the pathophysiology of Vasculitis.

**Recent Findings**—Substantial progress has been made in our understanding of the immunopathogenesis of 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. A new ANCA, directed against human lysosome membrane protein-2 (LAMP-2), has recently been described as a sensitive and specific marker for renal vasculitis and we discuss its potential impact for diagnosis and therapy. Furthermore, high throughput approaches are starting to identify genetic patterns that may identify patients likely to respond to specific therapy or having a high probability of relapse.

**Summary**—It has become increasingly clear, over the last two decades that ANCA IgG is pathogenic in vasculitis. 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 non-specific immunosuppression and its adverse effects.

**Keywords**

ANCA; vasculitis; autoimmune disease

**Introduction**

Autoantibodies specifically directed against neutrophils have been known to exist since the late 1950s and were first described in sera from patients with idiopathic leucopenia and later in Felty’s syndrome and rheumatoid arthritis. The vasculitis associated anti–neutrophil cytoplasmic antibodies (ANCA) were originally discovered by chance in the early eighties by Davies et al., in serum samples from patients with necrotizing glomerulonephritis [1] and have become the serological hallmarks of these diseases after van der Woude showed that they occurred in Wegener’s granulomatosis [2]. ANCA associated vasculitides are principally comprised of three overlapping, but distinct, disease entities, Wegener’s granulomatosis (WG), microscopic polyangiitis (MP) 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 [reviewed in 3**]. AAV has substantial morbidity and mortality, often presenting with aggressive renal failure or pulmonary haemorrhage, and requiring therapy with toxic immunosuppression [4]. Left untreated, AAV has a universally poor prognosis with mortality approaching 100% within 5 years [5]. The introduction of non-specific treatment regimens based on cyclophosphamide (CYC) and glucocorticoids (GC) have transformed AAV from a rapidly fatal disease, to one of chronic morbidity and reduced survival often preceded by end-stage renal disease (ESRD).

**ANCA specificity and pathogenicity**

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. ANCA are directed against the 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. greater than 95% of double positive patients with suspected nephritis reveal vasculitis on renal biopsy. PR3-ANCAs are detected mainly in WG, whereas MPO-ANCAs are predominantly found in MPA and CSS. The availability of ANCA testing has profoundly reduced the diagnostic delay of these conditions, but at the same time increased the median age at diagnosis and the incidence of vasculitis. Clinically, ANCA titres are frequently related to disease activity or relapse [6]. Therefore, a rising ANCA in a patient in clinical remission should raise suspicion of relapse and increase vigilance and follow up frequency.

A pathogenetic role of ANCA has been postulated since their close association with small vessel vasculitis was discovered. The most compelling *in vivo* evidence for the pathogenicity of ANCA in humans comes from a single case report of pulmonary hemorrhage and glomerulonephritis in a neonate with transplacental transfer of ANCA IgG from a mother with active MPO-ANCA vasculitis[7]. *In vitro* experiments show that ANCA induce neutrophil activation by engagement of their target antigens MPO and PR3. ANCA bind to neutrophils by Fc receptor engagement. This leads to neutrophil activation and release of oxygen radicals, lytic enzymes, and inflammatory cytokines, such as IL-8 [8]. This in turn impedes neutrophil migration [9] and results in excessive neutrophil accumulation within the vasculature and subsequent damage to the endothelium and vessel inflammation [10]. 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 10-fold increase in the number of transmigrating neutrophils [11, 12*]. Adhesion and migration require activation of neutrophil β2 integrins and involve the chemokine receptor CXCR2 [13].

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 [14]. A similar approach did generate PR3-ANCA, but passive transfer of these did not induce vasculitis [15], 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 [16, 17**]. The same study, explored the effects of MPO-ANCA on the induction of leukocyte-endothelium interactions using intravital microscopy of mesenteric venules [16]. Localised administration of the chemokine CXCL-1 (a rat homolog of interleukin-8) to the mesenterium of both immunized and naïve rats, pretreated with purified IgG from sera of MPO-immunized rats, led to increased leukocyte adherence, transmigration and focal hemorrhage 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. More recently, Little et al have extended their animal model experiments to examine different genetic strains in addition to the original WKY model. Despite the induction of significant ANCA titres, they were unable to replicate disease in 4 genetically distinct rat models (Lewis, Wistar Furth, Brown & Norway), thus alluding to a genetic basis for vasculitis resistance [17**]. Genetic preponderance is further illustrated by the induction of a vasculitis syndrome in SCID mice following the transfer of splenocytes harvested from NOD mice immunised with recombinant mouse PR3 but not following the transfer of splenocytes harvested in genetically distinct C57BL/6 mice and transferred to immunodeficient C57BL/6-RAG-1−/− mice [18*].

**ANCA mediated complement activation**

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 [19]. 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 [20]. Studies using mice deficient in specific complement pathways show that MPO IgG mediated glomerulonephritis is dependent on the alternative complement pathway [19]. 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 [21]. 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.

**ANCA and T cell activation**

T cells may play a major role in ANCA-associated vasculitides as ANCAAs are high-affinity, class-switched antibodies and their generation necessarily relies on T cells. T- cells localise to affected organs in patients with AAV and T-cell reactivity markers (eg, cytotoxic T lymphocyte-associated antigen) are increased in active disease[22].

Genetic studies and systems biology approaches are starting to shed some insight into the pathogenesis of AAV, in particular the role of T-cell subsets in disease activity and progression. McKinney et al employed gene expression assays to profile purified leukocyte subsets from 59 patients with AAV. Two distinct CD8+ T cell subgroups were identified, labelled v8.1 and v8.2. On clinical correlation, group v8.1 experienced significantly greater
A number of disease relapses during follow up (47.4% relapsed on more than one occasion in group v8.1 compared to 5.4% in group v8.2). The genes clustered in the poor prognostic group, v8.1, were further determined to have important roles in the interleukin-17 (IL-17) receptor pathway, T cell receptor signalling and memory T cell expression. Such prognostic information would likely inform therapeutic decisions, specifically the intensity of maintenance regimes required to reduce relapse. Interestingly, an identical sub-group was identified within a SLE cohort indicating more widespread applicability to auto-immune disease [23**].

The potential importance of adaptive immunity, specifically T memory cells and the IL-17 pathway, in disease relapse is further suggested by Nogueria et al. High levels of IL-17 and, its’ associated upstream cytokine, IL-23, were recorded in active AAV cases compared to healthy controls. Levels remained elevated, despite treatment, in a number of cases. Furthermore, IL-17-secreting autoantigen-specific memory T cells were detected during disease quiescence. This persistence is hypothesised to augment high relapse rates [24*], complementing the observations made by McKinney et al. This raises the possibility that ANCA associated vasculitis could indeed be a therapeutic target for pharmaceutical approaches targeting either IL-17 or Th17 cells.

**LAMP-2 a novel ANCA**

Literature examining the pathogenesis of ANCA associated Vasculitis (AAV), hitherto, has been dominated by myeloperoxidase (MPO) and proteinase-3 (PR3), two lysosomal enzyme ANCA antigens. A recent study advances the role of lysosomal membrane protein-2 (LAMP-2), another ANCA antigen. LAMP-2 is a type 1 glycoprotein which not only maintains lysosomal membrane integrity, but also contributes to key cellular functions such as adhesion, autophagy and antigen presentation. Autoantibodies to LAMP-2 were originally identified in the sera of patients with AAV renal disease over a decade ago [25]. Immunoblot experiments confirmed LAMP-2 as an ANCA target. Using a LAMP-2 ELISA, Kain et al have now reported a high prevalence of LAMP-2 autoantibodies in a cohort of patients with active pauci-immune focal necrotising glomerulonephritis (FNGN), the histological hallmark of ANCA associated renal vasculitis [26**]. Of the 84 patients, LAMP-2 antibodies were detected serologically in 78(93%). Furthermore, strong specificity is suggested by their absence in the sera of 53 healthy controls and 29 of 30 individuals with other forms of ANCA negative glomerulonephritis. Interestingly, however, 8 control participants diagnosed with other diseases, such as SLE, but known to possess antibodies to MPO, also tested positive for LAMP-2 antibodies. As with the canonical ANCA antigens, they are not 100% specific for the pathology. A close relationship with MPO and PR3 could be foreseen since LAMP-2 is expressed in the membranes of the intracellular vesicles where MPO and PR3 reside. On repeat testing following immunosuppressive therapy, LAMP-2 antibody became undetectable in 92% of patients originally positive. In addition, 20 cases of non-renal vasculitis were tested of which LAMP-2 antibodies were identified in only one sample. Both observations extend the possible future clinical applications of this potential biomarker from having a role in diagnosis, to becoming a measure of disease activity and disease prognosis.

Further reported LAMP-2 experiments have potentially greater implications, providing insight into the pathogenesis of AAV. A monoclonal LAMP-2 antibody was shown to induce neutrophil shape change and degranulation in vitro whilst also inducing apoptosis of vascular endothelium. The research group went onto induce FNGN by injection of LAMP-2 IgG antibody in 15 WKY rats, thus providing in vivo evidence for vascular pathogenicity. In order to further characterize LAMP-2, SPOTscan analysis was employed to identify LAMP-2 epitopes recognised by ANCA. A major sequence was located which was verified
to be identical to FimH, an adhesion molecule located on gram negative pathogens. Confirmatory reciprocal inhibition experiments showed cross reactivity between a recombinant FimH fusion protein and autoantibodies from patients with FNGN but not control sera. Finally, immunization of rats with FimH led to the development of LAMP-2 autoantibodies and the development of vasculitis syndrome. The authors conclude by suggesting that infection with fimbriated bacteria may result in the development of pathogenic LAMP-2 antibodies and consequent AAV renal disease through molecular mimicry.

While, this data has the potential to change the way these potentially fatal diseases are diagnosed, monitored and managed; replication and extension of these experiments in other centres is essential before moving forward. Many more questions remain unanswered, firstly it remains unclear how this novel ANCA antigen interacts with its more recognised colleagues, MPO and PR3. Secondly, why is the impact of LAMP-2 limited to renal disease alone? Thirdly, gram negative infections are common and ANCA associated vasculitis rare, so what host factors determine immune intolerance and progression to disease state.

Another study which supports the integration of infection into the AAV pathogenetic pathway examined neutrophil extracellular traps (NETS). NETS are chromatin fibres actively released by neutrophils. Their adhesive nature is thought to snare and then allow eradication of microbial invaders. They have also been shown to cause endothelial damage. There is previous evidence that *S.aureus* can strongly induce NETS [27]. Kessenbrock *et al* have now shown that NETs can also be induced by ANCA IgG and PR3-specific mouse monoclonal antibody but not control IgG. Furthermore, abundant levels of PR3 and MPO were localised to the chromatin fibres *in situ* and examination of renal biopsies with active disease revealed prominent NET accumulation *in vivo*. The authors concluded that NETS may be important instigators of vascular damage in AAV, a process which may be perpetuated by ANCA and bacterial infections [28*]. Again, host susceptibility may be key and an area requiring further investigation.

**Conclusion**

The search continues for less toxic and more effective modulation of the immune system using targeted immunotherapy. Substantial progress has been made in our understanding of the pathogenetic mechanisms that drive injury in ANCA mediated auto-immune disease. This opens the door to design novel therapies aimed at specific pathogenic pathways (complement, T- and B-cell activation, innate immune cell migration) raising hope to selectively treat this heterogeneous group of patients and diseases in the not too distant future.

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**List of abbreviations**

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<tr>
<td>ANCA</td>
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<td>AAV</td>
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<td>WG</td>
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<td>CSS</td>
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_Curr Opin Hematol. Author manuscript; available in PMC 2012 April 21._
MP  Microscopic polyarteritis
CYC  Cyclophosphamide
GC  Glucocorticoids
MPO  Myeloperoxidase
PR3  Proteinase 3
LAMP  Lysosomal Membrane Protein

References


