

## Genetics and Pathogenesis of small vessel vasculitis

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## **Abstract**

Small vessel vasculitides are uncommon autoimmune diseases, characterised by inflammation and necrosis of arterioles, capillaries and venules, commonly described as various (previously eponymous) clinical syndromes. Some are associated with vessel wall immune complex deposition while others are pauci-immune, but paradoxically often associated with circulating anti-neutrophil cytoplasm antibodies (ANCA). Most is known about the pathogenesis of the pauci-immune ANCA associated syndromes, which are gradually becoming better understood, with regards to their genetic predisposition and the critical pathways mediating disease initiation as well as their particular phenotypic features. Through better understanding of key cellular and molecular players we have been able to develop novel biomarkers and treatment strategies, which should translate to improved diagnostics, treatment protocols and ultimately better patient outcomes. These conditions are treatable, but not yet curable, although it is clear that patients may follow different disease courses, which for some include restoration of their pre-morbid immune status.

**Keywords: ANCA, immunity, vasculitis**

## **Introduction**

Small vessel vasculitides, characterised by necrotising inflammation in arterioles, capillaries and venules, may be divided into those associated with immune complex deposition, such as IgA Vasculitis (Henoch Schonlein purpura), cryoglobulinaemia and anti-C1q vasculitis, or those that are pauci-immune, without significant immunoglobulin deposition, including Granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg Strauss Syndrome) or microscopic polyangiitis (MPA), and these latter three conditions are frequently associated with a circulating anti-neutrophil cytoplasm antibody (ANCA)(1). Investigation into the pathogenesis of small vessel vasculitis is most advanced in ANCA associated vasculitis (AAV) which will form the main focus of this chapter, while application of genetics and molecular biology analysis should provide some greater understanding of the other immune complex conditions, such as IgA vasculitis. One of the great successes in the vasculitis field has come from the two way movement between laboratory science and clinical management, which has led to the testing of new therapies based on data from animal models, and the novel understanding of pathogenesis that has derived from the use of modern therapies such as B cell depleting agents. With such close links between the clinic and the laboratories, and with greater insights into key cellular and molecular players, there is a hope that further refinement of therapy will allow the diseases that were once almost universally fatal, to be not just be treatable, but one day be curable.

## **Autoantibodies and ANCA antigens**

Following the discovery of ANCA in patients with pauci-immune glomerulonephritis(2) and its subsequent close association with active GPA(3), the target antigens were identified as proteinase 3 (PR3) and myeloperoxidase (MPO). Tight correlations between PR3-ANCA expression and GPA are found but are less clear cut between MPO-ANCA and MPA(4). Both antigens are expressed in neutrophils and monocytes within specialised granules and lysosomes respectively, and are also

found decorating neutrophil extracellular traps (NETs) which are localised to inflammatory lesions within the kidney and other organs(5). Adaptive (B and T cell) immunity to PR3 and MPO have been shown in patients (see Immunity section below) with evidence for disease susceptibility incurred by certain HLA molecules (see Genetics). In addition differences in neutrophil and monocyte antigen expression have been identified. While both PR3 and MPO are expressed on monocyte cell surfaces, it seems that PR3 surface expression contributes to altered macrophage activation and a pro-inflammatory milieu, while binding of anti-MPO but not anti-PR3 antibodies to monocytes stimulate release of pro-inflammatory cytokines including IL-1 $\beta$ , IL-6 and IL-8(6). PR3 expression on the neutrophil surface is augmented in patients with GPA(7) and this leads to diminished clearance of apoptotic cells by macrophages(8), potentially allowing cells to undergo necrosis and release more antigen, provoking greater adaptive immune responses. Phagocytosis by macrophages of apoptotic, PR3-expressing neutrophils, results in secretion of pro-inflammatory cytokines such as IL-6, IL-8 and TNF- $\alpha$ (9). The greater expression is also found in unaffected relatives of GPA patients suggesting genetic regulation of antigen expression and SNPs in PRTN3, the gene coding for PR3 are associated with disease susceptibility in those patients with GPA or expressing PR3-ANCA (10). Interestingly, co-expression of PR3- and MPO-ANCA is rarely found, outside of particular polyclonal B cell stimulators such as certain drugs(hydralazine, propylthiouracil)(11) and following levamisole-contaminated cocaine use(12), suggesting that abnormalities of both autoantigen and HLA are required for specific autoimmunity to develop. Recent analyses of the MPO-ANCA during acute and remission phases of disease have suggested a shift from a predominant epitope reactivity during active disease to another during remission, potentially explaining the persistence of ANCA despite disease remission (13).

More recently, antibodies directed against other antigenic targets have been reported in subsets of patients but validation of their frequencies has been inconsistent and their roles in pathogenesis remain unclear. Whether they contribute to particular disease features (such as thromboembolic disease with anti-plasminogen antibodies)(14), augment disease severity (such as anti-moesin

antibodies)(15) or provoke disease under certain circumstances (following urinary tract infections with anti-LAMP2 antibodies)(16) needs further testing in larger, well phenotyped cohorts.

ANCA-negative disease is well recognised in a proportion of patients with limited renal or with ear, nose and throat (ENT) disease. Although often managed in a similar way to ANCA-positive disease, there is uncertainty as to whether these are the same conditions or simply share a final common pathway. Small cohorts of patients with ANCA-negative disease have been described and can present clinically as MPA, GPA, or renal limited vasculitis (RLV), with MPA predominating, and frequently with significant chronic renal damage on biopsy, possibly related to later diagnosis (17). Interestingly, while serum may be negative on ANCA testing, purified immunoglobulins from ANCA-negative patients may demonstrate reactivity to both whole MPO and the immunodominant B cell MPO epitope, suggesting that inhibitory molecules within the serum may prevent ANCA detection in standard immunoassays(13).

## **Genetics**

Although familial cases of AAV are reported, they are uncommon(18). However, like many other autoimmune diseases, both candidate gene approaches and genome wide association studies (GWAS) have implicated certain genetic loci as susceptibility factors for disease. In AAV two GWAS have demonstrated important associations between disease and the HLA gene locus on chromosome 6 in European and North American populations(10, 19). Differences are found in the HLA association depending on the clinical phenotype and the autoantibody specificity, with GPA and PR3-ANCA associating strongly with HLA-DP loci and MPO-ANCA associating with DQ loci(10). Previous small cohort studies have implicated HLADR B1501 in African American patients(20) and HLADP1 0401 is more frequently found in PR3-ANCA vasculitis and is as a risk factor for relapsing disease, regardless of ANCA subtype or clinical presentation(21). Although it remains to be proven, immunodominant areas of the autoantigens may have preferential binding to particular class II HLA antigens, and thus dictate the nature of the autoimmune response. In addition, PR3-ANCA was

associated with SNP's in the genes PRTN3 coding for PR3 and SERPINA1 coding for alpha1 anti-trypsin, one of the natural regulators of PR3. The particular biological impact of the SNP in PRTN3 remains uncertain however, one of the SNPs was associated with increased levels of PRTN3 transcripts in neutrophils (19). In addition, the SNP in SERPINA1 has been associated with altered levels of A1AT levels in patients (22). Other significant associations were found in PTPN22, a protein tyrosine phosphatase regulating T and B cell receptor mediated cell activation (19). The genetic associations suggest that disease could be better classified according to the antibody subtype rather than clinical phenotype, because the latter could change, for example from MPA to GPA, if certain clinical features subsequently developed. However, this leads to some problems when considering the significant minority of patients with ANCA-negative disease. It would be of great interest to examine genetic associations in the ANCA-negative patients, however, numbers needed are likely to limit the ability to perform such an analysis. A similar GWAS has recently been conducted in EGPA and has confirmed HLA associations shown by previous candidate gene approaches (HLA DRB4) as well as implicating other loci including IL-5(K Smith Unpublished). Recently a large cross phenotype meta-analysis of various vasculitis subtypes (including large vessel Takayasu's arteritis and giant cell arteritis and two small vessel vasculitides- IgA vasculitis and AAV), has confirmed a central role for HLA and an association with KDM4C (also known as JMJD2C), a gene encoding a histone demethylase, involved in epigenetic regulation of gene expression(23), suggesting a common predisposition for various forms of vasculitis. This is of interest because previous data have implicated alterations in methylation status of PR3 and MPO genes during active AAV, and altered levels of demethylase JMJD3 in AAV patients compared with controls(24).

### **Cellular immunity**

All immune system cellular components have been implicated in AAV, with a breakdown of tolerance to one or other of the autoantigens. Circulating ANCA are class switched immunoglobulins and require T cell help for their production, and both MPO- and PR3-specific T cells have been

identified in patients(25, 26). In addition, both B and T cells are found infiltrating tissues and are critical components in granulomatous lesions found in GPA (27-29). The balance of effector and regulatory T and B cells is impaired in AAV patients, with localisation and concentration of effector T cells in affected tissues during active disease, for example found in increased numbers in the kidneys(27) and urine of patients with active kidney disease (30). There are augmented antigen specific Th17 populations (31, 32) and diminished numbers and function of the regulatory T cells during disease remission (33-35). Animal studies have demonstrated immunodominant T cell epitopes for MPO (a peptide spanning amino acids 409-428 from murine MPO) which interestingly has an 8 amino acid overlap with the immunodominant disease associated B cell epitope recognised by the autoantibodies in patients during acute disease(36). Importantly, HLA transgenic mice immunised with the immunodominant B cell epitope demonstrated glomerular and urinary abnormalities and ANCA reactivity using murine neutrophils, while transfer of purified IgG from these mice to naïve animals also induced renal injury(13). B cells are implicated not just as precursors of autoantibody producing plasma cells, but also in antigen presentation, cytokine production, and promotion of pro-inflammatory T cells. Their central role in disease has been demonstrated by the success of B cell depletion agents such as rituximab(37, 38). However, B cells may also act to suppress autoimmunity and B cells with regulatory function (immature CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup>, or memory CD19<sup>+</sup>CD24<sup>hi</sup>CD27<sup>+</sup> cells) have been found to be functionally normal but numerically deficient in AAV patients during both active disease and remission, potentially contributing to the propensity for disease flare (39-41). By contrast patients with long term remission, off treatment, who have become ANCA negative demonstrate elevated levels of both regulatory T and B cells compared to relapsing patients(42), suggesting a more active regulatory phenotype contributes to disease quiescence. Macrophages and monocytes are found in affected tissue and circulating populations are altered in patients(6, 43, 44). These cells contribute to damage through release of reactive oxygen species and pro-inflammatory cytokines, and react to ANCA as they express both MPO and PR3, with one study showing increased levels of pro-inflammatory

intermediate monocytes (CD14<sup>hi</sup>CD16<sup>hi</sup>) which co-express highest levels of MPO and PR3(6) and another demonstrating elevated levels of CD14 in all PR3-ANCA patients and active MPO-ANCA subjects, again with a correlation between CD14 expression and PR3 and MPO expression on classical monocytes(CD14<sup>hi</sup>CD16<sup>neg/low</sup>)(44). However, it is the neutrophils that appear to be the main mediators of vessel damage, following their inappropriate activation, leading to a respiratory burst, release of extracellular traps(NETs) and proteolytic enzymes(45), resulting in endothelial damage(46). ANCA can lead to degranulation of cytokine primed neutrophils(47) and release of cytokines including B cell activating factor, found at high levels in patients with AAV and increased following rituximab treatment, promoting B cell survival(48). NETs are extruded from activated neutrophils, as strands of DNA decorated with neutrophil antigens (elastin, PR3, MPO, and calprotectin) and serve as effective anti-bacterial defences. NET formation is promoted by ANCA binding TNF-primed neutrophils in vitro and is dependent on the receptor-interacting protein kinase (RIPK) 1/3 pathway (46)while NETs are deposited in affected tissues during AAV(5). However, although levels of NETs are elevated in sera from active patients with both MPO- and PR3- ANCA associated disease, this effect persists following immunoglobulin depletion, suggesting an additional autoantibody-independent process (49). Interestingly, NETs may not contain equal quantities of PR3 or MPO, with PR3 being found predominantly in the cell bodies of neutrophils undergoing NETosis, while MPO appears contained within the NETs(50), while the particular trigger and neutrophil environment also influence the nature of the NETs formed (51). For example, propylthiouracil, with known potential to mediate MPO-ANCA vasculitis, appears to inhibit degradation of NETs and promotes an altered NET conformation in vitro and in vivo (52). Since NETs may be presented by dendritic cells to T cells, promoting antigen specific responses and ANCA formation (53), linking the innate and adaptive immune responses, different antigen containing NETs may help explain why double positivity for MPO-and PR3-ANCA rarely exists or indeed ANCA reactivity to either antigen with additional reactivity other neutrophil antigens, such as elastase or calprotectin. In addition,



NETs appear to provide a substrate on which alternative pathway complement activation can occur (46)(see complement below).

Neutrophil microabscesses form the nidus for granulomata formation, with multinucleate giant cells and subsequent recruitment of T and B cells(54), with evidence for local B cell activation and ANCA production(29, 55). These granulomata commonly form in the upper and lower airways, and less often in the retro-orbital space and kidneys. Although by definition they occur in GPA and not MPA, they can be found more frequently in European patients with PR3-ANCA, and in a minority of MPO-ANCA positive individuals (10). Animal models have remained elusive, although recent abstract reports in the murine anti-MPO model have shown intra-tracheal inoculation of lipopolysaccharide in animals given high dose anti-MPO antibodies appears to induce pulmonary granulomata(56). Overall, granulomatous disease remain difficult to treat and requires more prolonged therapy than vasculitic disease (57). The drivers for granuloma formation and the pathological basis of the close association with PR3-ANCA remain poorly defined.

While NET formation has not yet been used as a marker of disease activity, the infiltration of leukocytes into tissues has be capitalised on as a means of non-invasive monitoring of disease activity. Studies have demonstrated elevated urinary CD163 (macrophage) (58)or soluble CD25 (T cells/B cells)(59) expression being associated with active renal disease. The quest for a biomarker of future disease relapse to stratify immunosuppressive therapy remains, and may require combinations of immunoassays demonstrating failure to adequately suppress leukocyte activation once treatment has been started, and may include markers such as neutrophil calprotectin(60).

### **Complement**

Clear evidence of complement involvement in disease pathogenesis has emerged from animal and more recently patient data, in various populations. Using complement deficient mice and models of anti-MPO mediated vasculitis, a clear role for alternative pathway complement activation promoting disease was found, and specifically a role for the C5a receptor (C5aR) as a central component in

mediating these effects(61-63). This led to the development of therapeutic C5aR inhibition in patients, with a phase 2 trial demonstrating successful use of Avacopan, an oral C5aR inhibitor, as an alternative to oral glucocorticoids in a small selected cohort of patients (64). A larger phase 3 study in a more diverse population with more severe disease has recently completed recruitment and its results are awaited. In addition, altered levels of complement C3, C4 or CH50 are found in a minority of patients (5-20%) at presentation, but appear to be associated with worse clinical outcomes, such as overall patient and renal survival, incidence of pulmonary haemorrhage and more severe histological damage and immune complex involvement in renal biopsy specimens (65-67), with changes reported in European, Japanese and Chinese populations. In addition, C5aR has been shown both in vitro to prime neutrophils for activation by ANCA and in vivo its inhibition attenuates ANCA mediated glomerular neutrophil activation and retention while diminishing ANCA production, Th1 cell activation and promoting regulatory T cells(68).

## **Summary**

Small vessel pauci-immune, ANCA associated, vasculitis develops in genetically susceptible individuals with numerous immune phenotype variants (compared to healthy individuals) which lead to a breakdown in tolerance to a specific and limited repertoire of leukocyte antigens, resulting in particular clinical phenotypes. An intriguing question is whether or not we can re-establish immunological tolerance in those patients and revert back to a state of health, thereby preventing the relapsing-remitting disease cycle.

## **Practice Points**

Small vessel vasculitis may present in various ways and is part of a wide differential diagnosis.

Serological tests for ANCA, other autoantibodies, immunoglobulins, cryoglobulins and complement levels as well as histological assessment of the affected tissue are required to define the type of

vasculitis and whether it is pauci-immune or related to immune complex disease. Current concepts of pathogenesis suggest that stimulation of innate immune responses, possibly following infection, primes neutrophils and monocytes for aberrant activation by ANCA, in genetically susceptible subjects in whom autoreactive T and B cells are directed towards PR3 and MPO, and stimulate ANCA production.

### **Research Agenda**

Diagnostic delay remains a problem and ANCA remains the most useful screening tool. However, better and earlier diagnostics should be developed, perhaps incorporating use of artificial intelligence to learn the particular presenting features along with novel markers, incorporating genetic susceptibility ones, which may provide better means of picking up early disease. Secondly, although remission is achieved in the majority of patients with current treatments, there remains an issue of significant disease relapse, which results in the need for repeated cycles of immunotherapy and increased risk of adverse events. In addition, particular features such as granulomatous disease are especially difficult to treat, and better understanding of the drivers of granuloma formation should be pursued. Future investigations should be aimed at better understanding risk markers for relapse so as to customise therapy and define the required treatment duration. Ultimately resetting an abnormal immune response would be the ideal treatment that requires better understanding of how to attenuate the inflammatory and promote the regulatory immune circuits. Specifically,

1. Defining early diagnostic signatures for vasculitis
2. Understanding the basis for granuloma formation
3. Defining future biomarkers for disease relapse to enable stratification of therapy.
4. Developing better markers of subclinical inflammation that may persist in many subjects.
5. Trialling targeted therapies aimed at attenuating specific aspects of the pro-inflammatory response that may not be well regulated by current treatments such as Th17 cells
6. Understanding the immunological basis of long lasting remission.

## Figures

Figure 1. Small vessel vasculitides development due to immune complex deposition along the vessel wall or aberrant neutrophil activation in pauci-immune ANCA associated vasculitis

Figure 2 Neutrophil extracellular traps stained for a) DNA with DAPI and b) myeloperoxidase, formed following 4 hours of lipopolysaccharide stimulation of human neutrophils (x100)

Figure 3 Immune circuits in AAV demonstrating inflammatory and regulatory circuits that contribute to disease and its suppression. Autoantigens (PR3 and MPO) expressed on monocytes and neutrophils and present on NETs are presented to autoreactive T cells by antigen presenting cells including autoreactive B cells, stimulating T cell activation and polarisation with formation of pro-inflammatory Th17, Th1 and Th2 cells. These in turn can provide T cell help to B cells to form ANCA, which in turn can stimulate neutrophils and monocytes to release pro-inflammatory cytokines, including IL-1, IL-6 and IL-8 and BAFF, recruiting more leukocytes and inducing further neutrophil and monocyte activation and degranulation as well as NET formation, and promoting B cell survival. Regulation is impaired in patients, but achieved by regulatory T and B cells, inhibiting Th17 and Th1 cell proliferation and through the release of anti-inflammatory cytokines such as IL-10 and TGF $\beta$ .

Figure 1

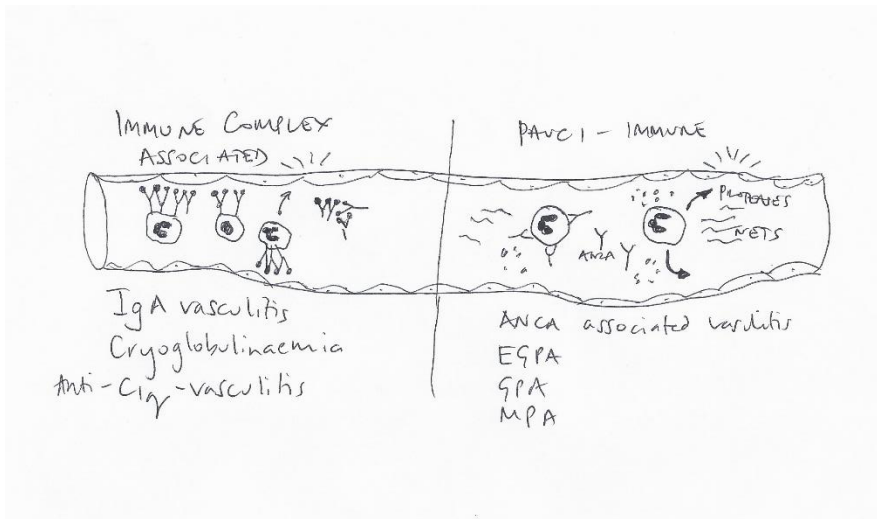
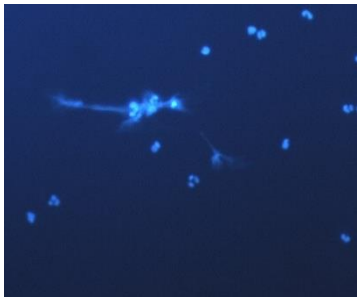


Figure 2

a)



b)

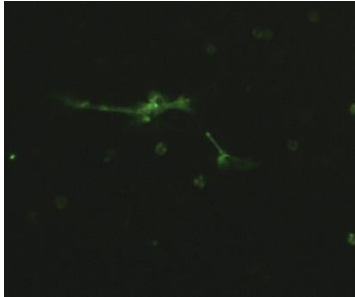
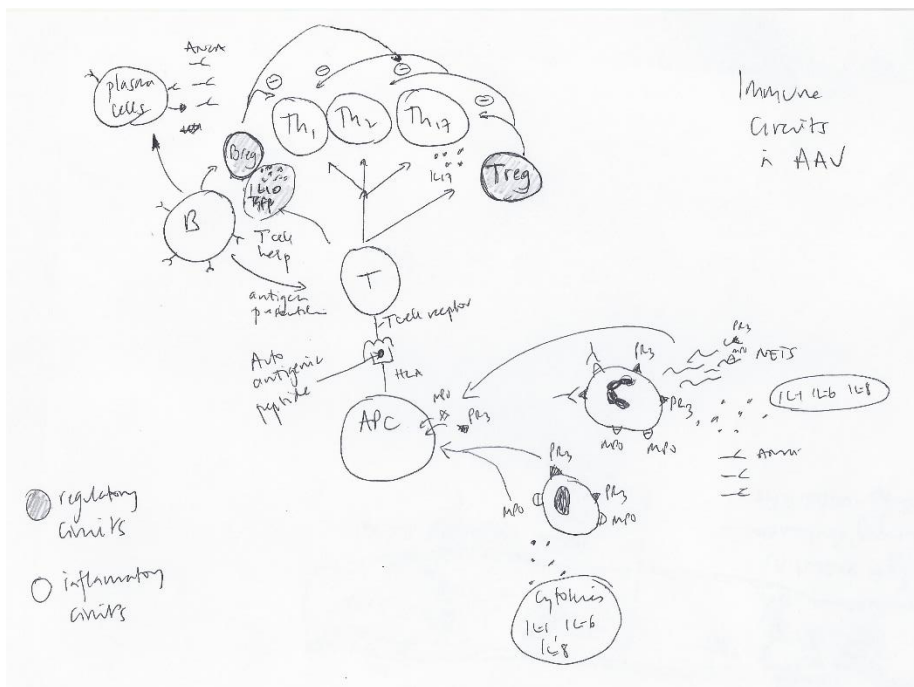


Figure 3



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