L1. Pathogenesis of ANCA-associated vasculitis: Observations, theories and speculations

Introduction
Antineutrophil cytoplasmic autoantibodies (ANCA) are an important class of autoantibodies that are associated with microscopic polyangiitis (MPA), granulomatosis with polyangiitis (Wegener’s) (GPA), eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA), and renal-limited vasculitis that manifests as glomerulonephritis indistinguishable from glomerulonephritis in MPA, GPA and EGPA. ANCA first became widely recognized as a result of a publication by van der Woude et al. in *Lancet* in 1985 that described circulating autoantibodies that reacted with antigens in the cytoplasm of neutrophils and monocytes in patients with GPA [1]. In fact, Davies et al. had previously described this class of autoantibodies in 1982 in a short report in the *British Medical Journal*, although their patients had features consistent with MPA rather than GPA [2]. Both articles touched on the issue of etiology and pathogenesis. Davies et al. suggested that the autoimmune disease might be triggered by infection [2] and van der Woude et al. proposed that these autoantibodies are pathogenic [1].

As with many other autoimmune diseases, the etiology and pathogenesis of ANCA-associated vasculitis (AAV) appear to be multifactorial and involve the interplay of initiating and predisposing environmental and genetic factors, loss of immune tolerance, mediation of acute injury, and response to acute injury (Figure 1).

Genesis of the antineutrophil cytoplasmic autoantibodies autoimmune response
Although Ehrlich proposed the dysteleology of autoimmunity (horror autotoxicus) and Burnet envisioned the elimination of forbidden clones, overwhelming evidence supports the presence of natural autoantibodies in all healthy individuals [3]. Thus, autoimmune disease is more a dysregulation of natural homeostatic autoimmunity rather than the onset of a previously absent state of self-recognition. Cui et al. showed that all healthy individuals have low levels of circulating natural autoantibodies specific for myeloperoxidase (MPO-ANCA) and proteinase 3 (PR3-ANCA) [4]. Using a sophisticated epitope detection system, Roth et al. have confirmed the presence of natural MPO-ANCA in healthy individuals and demonstrated that the epitope specificity of the repertoire of pathogenic MPO-ANCA differs from the repertoire of epitope specificity of natural MPO-ANCA [5]. Healthy controls have ANCA that react only with natural epitopes, patient in remission have ANCA reactive with natural and nonpathogenic epitopes, and patient with active disease have ANCA reactive with natural, nonpathogenic and pathogenic epitopes [5]. Thus, not all ANCA are equal, and the ANCA autoimmune response must have a particular repertoire of autoantibodies to mediate injury.

The transition from natural autoantibodies to pathogenic autoantibodies involves both quantitative and qualitative changes in immune regulatory mechanisms, especially mechanisms mediated by regulatory T cell (Tregs) and regulatory B cells (Bregs) [6]. There is evidence for defective Treg function in patients with AAV [6,7]. Meghan Free et al. have demonstrated that circulating Tregs are increased in AAV patients with active disease but these cells have decreased suppressive function because they utilize a FOXP3 isoform that lacks exon 2 [7]. In addition to defective Treg function, patients with active AAV have an expanded antigen-experienced CD4+ T cell population that is resistant to Treg suppression. B cells as well as T cells can be involved in the regulation of immune responses, and there is evidence that there is a reduction of regulatory B cells during active AAV [8]. Thus, ineffective T cell and B cell regulation may predispose to and sustain loss of tolerance and pathogenic ANCA production.

The etiology for the pathogenic ANCA autoimmune response is unknown. Infections have been incriminated, beginning with the suggestion by Davies et al. that infection with Ross River virus may have induced ANCA in his patients [2]. *Staphylococcus aureus* has long been known to be associated with GPA [9], although the precise immunologic link has not been proven. One intriguing possibility was proposed by Pendergraft et al. who described observations in patients with PR3-ANCA disease supporting the theory that peptides that are complementary to the autoantigens are the initial impetus for evolution of the pathogenic antibodies directed against the autoantigen [10]. Complementary peptides are the products of the transcripts of anti-sense DNA opposite the sense DNA. Peptides from other sources can mimic anti-sense peptides. Anti-sense peptides occur naturally [11] and thus an immunogenic complementary peptide could be endogenous.
Alternatively, an exogenous mimic of a complementary peptide could be brought in by an infectious pathogen. Interestingly, both *S. aureus* and Ross River virus have peptides that mimic PR3 anti-sense complementary peptides [10]. The theory of autoantigen complementarity posits that an initial antibody response (A1) to an anti-sense peptide or a mimic that acts as a complementary peptide (Ag1) stimulates an anti-idiotypic response to the A1 idiotope that produces A2 antibodies that cross-react with the autoantigen (Ag2) (figure 2, upper left panels).

In addition to infections, other environmental exposures can influence the development of ANCA-associated vasculitis. For example, Hogan et al. used a population-based, case-control study to document that silica exposure is associated with AAV [12]. Extensive exposure to silica with resultant accumulation in cells of the reticuloendothelial system may have an adjuvant effect on antigen presenting cells, especially in the respiratory system, resulting in more effective presentation of ANCA autoantigens for initiation of an autoimmune response.

Genetic predisposition influences the onset and mediation of AAV (figure 1). For example, a genomewide association study implicated a genetic influence on the pathogenesis of AAV and that this differed between MPO-ANCA and PR3-ANCA disease [13]. PR3-ANCA AAV had 73.3-fold higher odds of having HLA-DRB1*15 alleles than healthy controls [14]. A disproportionate number of African American patients carried the DRB1*1501 allelic variant of Caucasian descent rather than the DRB1*1503 allelic variant of African descent. Interestingly, DRB1*1501 protein bound with high affinity to amino acid sequences of both sense-PR3 and anti-sense (complementary) PR3, which supports a role for MHC ABS specificity in disease induction.

Epigenetic as well as genetic factors can influence pathogenesis. For example, Ciavatta et al. observed epigenetic modifications in ANCA autoantigen-encoding genes that result in increased expression of PR3 and MPO in neutrophils of ANCA patients (figure 2, upper right panel) [15]. This could predispose to both induction and mediation of ANCA disease by providing greater amounts of target autoantigens.

Thus, genetic predisposition, environmental factor and disturbances in immune regulation all conspire to set the stage for initiation of the pathogenic events that cause AAV.

**Mediation of vascular and extravascular inflammation**

Figure 3 depicts putative microenvironmental pathogenic events that cause acute vascular inflammation [16]. Beginning at the left of the diagram, neutrophils (green) that have been primed with cytokines release enough ANCA-antigens (e.g. MPO or PR3) at the surface and in the microenvironment to cause activation of neutrophils by ANCA. This involves both Fc receptor engagement and Fab’2 crosslinking. ANCA-activated neutrophils release factors that activate the alternative complement pathway, which generates C5a that amplifies the inflammation in multiple ways, including recruitment of...
more neutrophils and priming of neutrophils for ANCA-activation. The result is acute necrotizing injury to vessel walls. Extensive in vitro evidence supports this scenario, including the priming of neutrophils with cytokine for activation by ANCA [17,18], initiation of activation pathways by both Fc and Fab’2 engagement [19–21], release of ANCA-antigens into the microenvironment for interaction with ANCA [22,23], and injury of endothelial cells by ANCA-activated neutrophils [24–26]. Although neutrophils may be the dominant effector cell of acute injury, it is important to note that monocyte also contain MPO and PR3 and can be activated by ANCA [27–29].

Multiple animal models support the pathogenic scenario in figure 3. Convincing animal models of MPO-ANCA disease have been developed in mice and rats [30]; however, a robust animal model of PR3-ANCA disease has not yet emerged although several possible models have been described [31–35]. The first conclusive model of AAV is induced by injecting murine anti-MPO IgG intravenously into mice, which results in necrotizing and crescentic glomerulonephritis and vasculitis within 6 days [31]. Studies using this model, or variants of this model, have demonstrated that the vascular inflammation is caused by anti-MPO IgG alone and not T cells, neutrophils are required, circulating cytokines exacerbate

disease, alternative pathway complement activation is required, FcγR are involved in pathogenesis and disease modulation, the structure and specificity of anti-MPO influences pathogenicity, and genetic factor modulate disease susceptibility and severity [36–45]. Little et al. also have demonstrated the pathogenicity of anti-MPO using a model that involves immunizing rats with human MPO, which results in circulating anti-MPO that cross-reacts with rat MPO and causes glomerulonephritis [46,47].

Activation of the alternative complement pathway has emerged from the animal models studies as an important amplification step in ANCA-induce inflammation (figure 3) [40–42]. Subsequently, the importance of complement in patients with AAV has been supported by the presence of activation components of the alternative complement pathway in plasma and inflamed tissue of patients [48–50]. Models of ANCA disease have focused on the induction of necrotizing and crescentic glomerulonephritis and, to a lesser extent, on small vessel vasculitis, but have not shed much light on the extravascular necrotizing granulomatosis of AAV that occurs in GPA and EGPA. In our judgment, there has been substantial confusion about the nature of the granulomatous inflammation in GPA and EGPA, which has influenced theories about its pathogenesis. Most theories presume that this inflammation is analogous to T cell mediated granulomatous inflammation such as that seen with tuberculosis or sarcoidosis. However, the necrotizing granulomatous inflammation of GPA and EGPA begins not as an accumulation of predominantly T cells and macrophages, but rather as an accumulation of neutrophils resembling a microabscess [51]. One hypothetical (speculative) mechanism for the initiation of necrotizing granulomatosis is the activation of neutrophils in the extravascular tissue by ANCA (figure 4). In a patient with circulating ANCA, there also is interstitial fluid ANCA in the extravascular compartment. If the extravascular compartment contains neutrophils, for example because of a synergistic infectious or allergic process, these neutrophils would be susceptible to activation by ANCA. ANCA-activated extravascular neutrophils would recruit more neutrophils (e.g. by alternative complement pathway activation) resulting in intense necrotizing inflammation. This tissue injury would engender an innate inflammatory response that would attract monocyte that would transform into macrophages, which in turn would recruit T cells. With persistence of the central zone of debris, the response to injury would evolve toward more typical granulomatous inflammation with peripheral macrophages (including giant cells) and lymphocytes (predominantly T cells) (figure 4).

In unpublished studies, Xiao et al. have observed that anti-MPO can cause severe extravascular granulomatous inflammation in mice if they have predisposing characteristics of their innate immune system and synergistic inflammation. For example, if...
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healthy wild type B6 mice are injected with anti-MPO IgG, less than 30% have pulmonary alveolar capillaritis affecting less than 5% of the lung parenchyma and only vary rarely having a granulomatous component. If mice deficient in the inhibitory Fc gamma receptor Iib are given the same dose of anti-MPO, approximately 90% develop alveolar capillaritis and half of these have a component of granulomatous inflammation. And if mice deficient in Fc gamma receptor Iib are first infected with influenza virus to produce parenchymal inflammation and then injected with anti-MPO, 100% develop severe pulmonary disease with a granulomatous component in approximately 80% (Xiao et al, unpublished data). These three conditions caused by the same dose of anti-MPO IgG result in three different disease manifestations that resemble renal-limited disease, MPA and GPA respectively. The differences are caused not by differences in the pathogenic antibodies but by differences in the innate immune response and the presence of synergistic inflammation. These observations support the concept that the granulomatous inflammation of AAV results from extravascular ANCA-induced neutrophil activation in tissue in the setting of a synergistic inflammatory milieu.

As with all acute injury, the innate immune response to injury mediates resolution or chronic progression of the vascular and extravascular ANCA-induced inflammation. Repeat biopsies of dermal leukocytoclastic angitis demonstrate that neutrophil-rich acute inflammation is replaced by predominantly monocytes and T lymphocytes within a few days. Unpublished observations by Hong Xiao and associates show that the acute segmental necrotizing lesions of anti-MPO induced glomerulonephritis evolve into segmental sclerotic lesions within only 1 week. It is important to recognize that the chronic inflammation and scarring the follow the acute injury may be very important in the clinical outcome and thus should be taken into account in any treatment regimen.

**Implications for treatment**

Because the etiology and pathogenesis of AAV is multifactorial and involves the interplay of multiple factors at multiple stages of the disease, optimum therapy likewise must be multifaceted and directed at multiple elements of this complex pathogenesis, and will be different at different stages of the disease. For example, the pathogenic autoimmune response could be suppressed or redirected with immunomodulatory therapy (e.g. anti-B cell therapy or adoptive transfer of Tregs), the acute necrotizing injury could be ameliorated with anti-inflammatory therapy (e.g. corticosteroids and complement inhibition), and the chronic response to injury could be diminished by anti-fibrotic therapy (e.g. angiotensin-converting-enzyme inhibitors). Undoubtedly, a more thorough knowledge and understanding of etiologic and pathogenic events will enable more effective treatment of AAV.

**Disclosure of interest**: Dr. Jennette is a consultant for Genentech Inc.

**References**


[3] Jennette JC, Falk RJ. The rise and fall of horror autotoxicus and forbidden bodies but by differences in the innate immune response and the presence of synergistic inflammation. These observations support the concept that the granulomatous inflammation of AAV results from extravascular ANCA-induced neutrophil activation in tissue in the setting of a synergistic inflammatory milieu.


Neutrophils are a major component of innate immunity and indispensable for host defence against invading pathogens [1]. Due to their tissue destructive ability, neutrophils are also intimately associated with the development of acute inflammatory disorders such as ARDS and myocardial infarction. Traditionally, neutrophils have been considered as short-lived “kamakazi” cells that arrive rapidly at sites of infection and injury, are over-exuberant in their activity, and die within the infiltrated tissue. Growing body of evidence has however challenged this dogma suggesting that neutrophils may have a longer life-span than previously considered (e.g. 5.4 days in humans) [2], can be detected in lymphoid organs [3], are associated with the pathogenesis of numerous chronic inflammatory disorders and exhibit elaborate interactions with components of the adaptive immune response [4]. Emerging evidence also indicates the presence of different neutrophil sub-sets with distinct phenotypic and functional profiles in various disease scenarios [5]. Furthermore, contrary to being dead-end cells, neutrophils can exhibit reverse transmigration [6–8], a phenomenon that recent studies stemming from our laboratory has associated with development of systemic inflammation [9] (see below and Figure 1). Collectively, current evidence indicates a broader role for neutrophils in inflammation, immunity and pathogenesis of inflammatory disorders than conventionally considered, emphasising the need for greater insight into the mode, mechanisms and implications of neutrophil trafficking in vivo.

In this regard, using an advanced confocal intravital microscopy platform, we have analysed details of neutrophil-vessel wall interactions in real-time in 3D and have noted previously unreported physiological responses (e.g. sub-endothelial cell crawling [10]) and also potential pathological events such as “disrupted” modes of neutrophil transendothelial cell migration (TEM) [9]. The latter includes the first direct observation of in vivo neutrophil reverse TEM (rTEM) within a mammalian system. In investigating the pathophysiological relevance of this event, we noted that neutrophil reverse TEM was most prevalent following ischemia-reperfusion (I-R) injury and was associated with the presence of a subset of functionally primed neutrophils within the pulmonary vasculature which correlated with the development of acute lung inflammation [9]. These results suggest that neutrophils that have undergone reverse TEM have a distinct and activated phenotype, and as a result, may contribute to turning a local inflammatory response into a systemic multi-organ phenomenon (Figure 1). As the above findings have identified a potentially new paradigm in dissemination of systemic inflammation, and since there is evidence...