The Pathogenesis of Antineutrophil Antibody Associated Vasculitis: Environmental and Genetic Considerations

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Abstract

The review seeks to summarise the current ideas in regard to the pathogenesis of ANCA-associated vasculitis and to examine in detail how epidemiological and genetic factors fit with the modern paradigm. The recent literature has been reviewed. The AAVS appear to involve initiation by both T cells and B cells followed by a neutrophil dominated inflammatory phase in which ANCA may actually be involved. The alternative complement pathway may play a role. The genetic background is reviewed with genes identified that potentially encode proteins that are involved in the regulation of the immune system, other genes may be involved in the control of the inflammatory phase. With regard to environmental factors the two that stand out are a latitude gradient, presumably vitamin D and silica an agent known to be associated with both autoantibody production and autoimmune disease. A model which includes these factors is outlined.

Keywords: Vasculitis; ANCA; Genetics; Environment; Pathogenesis

Introduction

The Antineutrophil Associated Vasculitides (AAV) are widely regarded as autoimmune diseases although the role of antigen specific autoimmune mechanisms; antibody reactivity to neutrophil derived antigens has only been described relatively recently [1]. The diseases include granulomatosis with polyangiitis(GPA), Microscopic Polyangiitis (MPA) and Eosinophilic Granulomatosis With Polyangiitis (EGPA) although the latter may well include a form of the syndrome without autoantibodies [2] and perhaps general points made in this opinion article are less relevant to ANCA negative EGPA. Autoimmune diseases are generally considered to involve a complex interplay of environmental and genetic factors. Genes, generally with the capacity to influence the way in which the immune system operates have been identified [3,4] but with the exception of certain drug induced syndromes [5] and gliaden induced enteropathy [6] the nature of most environmental agents remains unclear [5]. Although there is a lack of knowledge about precise triggers of most autoimmune diseases considerable information has accumulated about potential environmental cofactors that may bias the immune system to auto reactivity [7].

Pathogenesis of AAV

This has recently been well reviewed by others [8,9] and can be usefully considered to involve several steps, initially perhaps in a particular sequence, although once the disease is well established it is not really known if all mechanisms operate together or if the original triggers no longer necessary. AAV can be considered to have an initiation phase where immune tolerance is broken and auto reactive T cells and B cells expand and exert their effect. This is most obviously expressed by the development of ANCA, but at the very least because of the need for T cell help to make high affinity IgG autoantibodies auto reactive T cells are also present. Indeed, typical pathology of the AAV is described as pauci-immune raising the prospect of a greater role for T cells than mere help for antibody synthesis [8,9]. The autoantibodies of particular interest include anti-proteinase 3 (anti-PR3) associated mainly with GPA and anti-myeloperoxidase (anti-MPO) associated with MPA and a proportion of EGPA. In regard to lymphocyte auto- reactivity the first issue to consider is whether or not ANCA are in any way directly pathogenetic. The evidence supporting a pathogenetic role for anti-MPO has been summarised [10] and is quite strong and generally accepted. The evidence that anti-PR3 are pathogenetic is not nearly as strong, although the level of expression of PR3 appears important, consistent with it being a target. Evidence for a role for various subsets of T cells in the pathogenesis of all 3 forms of AAV is compelling with expansions in both TH1 and TH17 cells as well as their respective cytokines noted in several studies. This indicates at least two pro-inflammatory effector T cell pathways are operational in AAV. In particular TH17 cells can increase production of and activate neutrophils providing a direct link to the effector phase of the disease [8,9]. Parallel defective function of TREGS has also been reported as well as a persistent deficiency in mucosal-associated invariant T cells(MAIT) consistent with a role for impaired cellular regulation [11]. Helper T cells, TH2 cells also appear to be activated, as indeed would be expected as the ANCA are high affinity IgG antibodies. Recently evidence has accumulated for a relatively novel pathway involving IL33 a member of the Il1 family interacting with its receptor ST2 in a complex manner involving both surface receptor ST2 on TH2 cells and soluble ST2 which competes for IL33. IL33 is probably produced from vascular endothelium, in response to inflammatory stimuli, including neutrophil degranulation and may thus be a feed forward loop. Soluble ST2, but not IL33 is elevated in active AAV although clearly what is important is what occurs at a tissue level [12]. This initiation phase may be followed by a phase of consolidation and amplification of the autoimmune response, but longitudinal studies have been insufficient to be certain of this. Some patients however have delayed and muted progression despite persistent and high titres of MPO-ANCA [13] or gradually increasing...
titres of PR3-ANCA [14], both consistent with the need for another step before disease is inevitable.

Finally there is an effector phase when major tissue damage occurs. *In vitro*, ANCA stimulate primed neutrophils to undergo a respiratory burst, degranulate and release toxic proteins and adhere to endothelium. *In vivo* MPO-ANCA may do this, but there is only limited evidence supporting the pathogenicity of anti-PR3 with no examples of placent transfer leading to disease and no convincing animal models to date [8,9]. Detailed analysis of epitope specificity with MPO-ANCA indicate an evolution with time to more readily detectable and pathogenetic ANCA [8,9]. Furthermore in remission the number of epitopes recognised decreased [15,16]. There is some evidence that epigenetic factors may underpin an increased expression of either MPO or PR3 enhancing the neutrophil reactivity [9].

AAV all appear to involve a stage of activation of neutrophils, probably monocytes and in the case of EGPA eosinophil. ANCA probably participate in the activation of neutrophils, but also require non-specific inflammatory stimuli such as might arise from exposure to microorganisms. The antigens PR3 and MPO are expressed on the surface of neutrophils amplifying the reaction. The activation of inflammatory cells initiates tissue damage and leads to vascular wall damage and vasculitis. Activated neutrophils extrude neutrophil extracellular traps (NETS). This normal defensive trap for bacteria appears to participate in the inflammation of AAV by releasing potential auto antigens and enhancing the antibody response.

Despite AAV being pauci-immune, that is immune complex poor further studies have detected C3 deposits in over half of a series of renal biopsies. Animal models show C5 deficiency or inhibition protect against vasculitis. C5 products are activators of neutrophils and at the very least an amplification pathway could exist. Further analysis of the alternative complement pathway, including genetic studies in AAV are warranted [8,9].

**Environmental Factors**

**Epidemiological studies**

It is appropriate to briefly review these as they have been detailed in previous publications [17,18] and although further population based studies have been reported no new risk factors have been identified. They can be summarised as follows:

1. The AAVS occur with advancing years with GPA peaking in the middle aged to elderly with a variable but small ratio of males to females).
2. AAV, in particular GPA are diseases of Caucasians of European origin. This has been reported from France, the United States of America (USA) and New Zealand (NZ).
3. There is evidence suggesting an increase in incidence of AAV over the last several decades, although this seems to be plateauing out.
4. Fluctuations over time, seasonal differences and urban versus rural patterns exhibit no consistent differences.
5. A latitudinal gradient exists for GPA in both northern and southern hemispheres. A similar but weaker gradient exists for EGPA. The relationship is in fact much stronger with ambient ultra-violet radiation (UVR), particularly winter radiation. The most plausible explanation for these findings is that it is due to effects of low vitamin D on the immune system [19]. Unfortunately population data on vitamin D levels in AAV or indeed populations from which such subjects are drawn is lacking (Table 1).

**Case Control Studies**

The dominant risk factor in the nine case control studies is silica, both crystalline silica and silica found in crop dusts [17,18]. Any role of air pollution appears to largely be silica exposure. Silica exposure is reported many autoimmune diseases and in AAV includes both pulmonary and extra pulmonary manifestations [20]. Case control reports also lack any data about personal UVR exposure or vitamin D levels.

**Infection**

The role of infection in AAV could be to provide a causative trigger or a trigger for relapse or both [21]. Although this may include chronic or latent viral infection most evidence to date supports a potential role for bacterial infection. A correlation between increased nasal carriage of staphylococci, including specific types and relapse has been reported [22,23]. The mechanism of action of Staphylococcus remains to be elucidated.

There is evidence for molecular mimicry between the human LAMP-2 epitope (a common ANCA specificity, lysosomal membrane associated protein 2) and bacterial adhesion FimH derived from many gram negative organisms [24]. An animal study with rats immunised with FimH developed cross reacting LAMP-2 antibodies and pauci-immune GN [11,25]. This quite compelling hypothesis remains in limbo as both the animal model and serological studies have proved difficult to repeat in other laboratories [11].

**Drugs and Toxic Chemicals**

There are specific associations with medications and AAV including propylthiouracil (PTU) and hydralazine which can trigger both ANCA and less commonly AASV [26,27]. EGPA has been linked to all forms of leukotriene receptor antagonists (LTRA). These medications may have been used for severe asthma which was part of the onset of EGPA, but probably not in every case [28]. Erythromycin like antibiotics have also been associated with EGPA [29-31]. In one case there was confirmed with a positive repeat challenge [31]. No single disease mechanism emerges from these studies and the risk of EGPA with these medications appears low and uncertain.

**Genetics of AAV**

Reports from three separate countries clearly show a predominance of white Caucasians over other ethnic groups sharing the same geographical entity suggest that genetics may be important. In contrast, unlike many other autoimmune syndromes familial clustering appears to be quite rare [32]. This subject has been recently reviewed in a meta-analysis and this together with a genome wide association study (GWAS) has strengthened genetic associations [33,34]. The authors reported 33 genetic variants associated with AAV. The variants were in or near the following genes: CD226, CTLA-4, FCGR2A, HLA-B, HLA- DP, HLA-DQ, HLA-DR, HSD17B8, IRF5, PTPN22, RING1/RXRB, RXRB, STAT4, SERPINA1, TLR9 (Figure 1). The more impressive associations included SERPIN 1A which encodes a protease inhibitor important in controlling excess damage mediated by neutrophils. The S and the Z allele are associated with both PR3-ANCA and MPO-ANCA. There are MHC class II linkages; DBP1*0401 with GPA and
MPA in Caucasians and DRB1*0901 with MPA in Japanese and DRB4 with EGPA. The strongest risk linkage was with HLA-DPB1*0401 with a modest odds ratio of about 2 [34].

Figure 1: Schema of the immune pathogenetic mechanisms for the AAV. The role of genetic factors and environmental factors is indicated. Clearly there is much to be filled in. Details in text.

Furthermore recent studies have shown that this variant is associated with a greater tendency to relapse, even more so if the patient is homozygous [35]. There are clear ethnic differences here as would be expected and there are some interesting findings. An association has been found between HLA-DRB1*0401 and MPA in Japanese and DRB4 with EGPA. The strongest risk linkage was with HLA-DPB1*0401 with a modest odds ratio of about 2 [34].

Table 1: Summary of both genetic and environmental risk factors for the three different syndromes of AAV. In general where studied genetic risk factors are stronger with the serology than the clinical diagnosis. The details are contained in the text. Y=yes, N=no

- MHC
- CTLA-4
- PTPN22
- Antigen expression Y-genetic Y-epigenetic
- SerpinA1
- Silica
- Low UVR/vitamin D

The initiation phase of these diseases is the likely site of involvement of the MHC, presumably permitting a pathological response against neutrophil derived antigens. Whether this involves molecular mimicry, hidden epitopes or some other mechanism is unclear. Class II MHC is involved indicating a role for CD4 T cells. T cells probably play a role as effector cells in the pauci-immune lesions that characterise AAV. This is also supported by the recurrence of both GPA and MPA after B cell depletion with rituximab with no return of ANCA [36]. Indeed there is evidence to support a role of TH17 effector T cells and evidence for less effective TREGS [37]. Nevertheless there does appear to be a role for at least anti-MPO as indicated most convincingly by trans placent transfer and neonatal MPA [10]. Clearly, TH2 cells are also involved [12]. Additional risk genes encode regulatory proteins that would be manifest in overactive effector T cells or underactive TREGS modifying the autoimmune response accordingly as it develops. Encoded allelic differences in those discovered so far, CTLA-4 and PTPN-22 would operate at a T cell level. In addition the gene encoding PTPN-22 may help explain some of the major ethnic differences. The allele increased in GPA is quite common in White Caucasians and quite rare in other ethnic groups, consistent with the observed occurrence. The findings in regard to PR3 in the GWAS provide further support for the importance of this protein as an autoantigen although whether or not its expression is increased in GPA is unclear. Once a T cell initiated and driven response arises there is progression to an inflammatory effector phase dominated by neutrophils and genes that would enhance neutrophil activity such as those encoding dysfunctional alpha-1-antitrypsin, the SERPIN1A1 locus would to play a role here. This accumulation of relatively weak genetic influences combines with the concept of quantitative thresholds for immune-cell signalling which alters lymphocyte function and allows an understanding of how multiple genetic factors of a relatively weak individual effect can combine to magnify the effect
The environmental observations show both overlap between syndromes in that silica exposure is a risk factor for both GPA and MPA but also clear differences with drugs being associated particularly with MPA and in the case of another group of drugs EGPA. The association between GPA and EGPA and ambient UVR, most probably vitamin D is not shared with MPA. A potential explanation for these findings is to separate the pathogenesis of granulomas, seen in GPA and EGPA from necrotising vasculitis involving small arteries, arterioles, capillaries and venules, common to all three syndromes. Current evidence suggests that the vasculitic lesions involve neutrophil activation consequent neutrophil enzyme and other product mediated vascular damage [10,11]. Neutrophil recruitment may be driven in part by TH17 cells where antibody production is really integral to the development of vasculitis then TH2 cells are presumably involved [10]. Monocytes are also involved and eosinophils in EGPA [2,10]. The development of granulomas may involve a number of different pathways although traditionally they are considered a product of disturbed innate and acquired cellular immunity and in the context of GPA and potentially EGPA with overactive TH1 and TH17 cells and a reduction in numbers and/or activity of TREG cells [37,38]. Low vitamin D would produce its effect through decreased TREG activity [39] and subsequent increased TH1 and TH17 activity. This fits with the latitude gradient seen with both GPA and EGPA, but not MPA where there are no granulomas [10,11]. Silica exposure produces multiple immune effects. This includes decreased TREGs and increased TH17. Silica binds to the neutrophil peptide LL-37 and induces ANCA antigen synthesis, acting as an environmental cause of increased antigen expression, to potentially prime the autoimmune reaction or act as a target or both [40].

The role of infection may include a non-specific pro-inflammatory adjuvant effect and/or specific triggering by particular agents such as Staph aureus or gram negatives [11]. Infection could certainly provide or drive production of the innate stimuli that act as cofactors to neutrophil activation. The favourable effect in EGPA of any therapy that decreases eosinophil suggests that this cell plays a pivotal role in the pathogenesis. An additional TH-2 cell mechanism includes IL-5 mediated T cell control. Effector T cells, TH1 and TH17 have been reported and could drive the granulomatous component of the disease [41]. In conclusion growing knowledge about both genetic and environmental associations with the AAV is allowing a plausible understanding of the disease pathogenesis. Other areas of current interest in autoimmune disease such as the gut microbiota, psychological stress, physical activity and the metabolic syndrome all remain to be studied in the context of AAV.

References