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

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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) and 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 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...
Epidemiological studies

Environmental Factors

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 AASV 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 AAV [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, PTSP22, RING1/RXXB, RXXB, STAT4, SERPINA1, TL9B (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; DPB1*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*15 allele in African Americans and PR3-ANCA related disease. This was particularly the DRB1*1501 variant which is of Caucasian origin as opposed to the DRB1*1503 African variant [11] As with HLA-genes other susceptibility genes encode proteins that play a role in the acquired or innate immune systems. Other susceptibility genes affect the putative autoantigens in either a qualitative or quantitative fashion. Understanding the mode of action of gene products is the key to understanding the clues provided by genetic findings. Genes that encode proteins known to be important in regulation of T cell activity have been detected repeatedly, usually with weak odds ratios of 2-3. A less efficient form of the negative regulator protein CTLA-4 which would be associated with persistent immune activity has been demonstrated. In contrast, an allelic variant Lyp*W620 of lymphoid tyrosine phosphatase (PTPN-22) encoded by the gene (T1858) is increased in GPA and MPA. This should deliver less effective regulation of T cell activity and there remains some contention about the mechanism of this protein in human cells [34]. The results of the GWAS confirm the association in white Caucasians of HLA DBP1*0401, the SERPINA1 locus which encodes alpha-1-antitrypsin, a regulator of neutrophil function and added the PRTN 3 locus which encodes the antigen PR3. A weaker association between MPO positive subjects and HLA-DQ was shown. Perhaps the most important finding from the GWAS was the demonstration that all associations were stronger with the serological profile, anti-PR3 and MPO than with the clinical diagnosis [33].

**Synthesis**

In drawing these factors into a current plausible model for these 3 diseases it must be recognised that despite their potential similarities there almost certainly are distinct etiopathogenetic pathways in the different syndromes [10,11]. It is certain that present knowledge is far from complete any synthesis remains speculative. In particular, although current very reasonable overviews of the diseases are overarching and inclusive of all forms of AAV, or at least GPA and MPA, the genetic and environmental differences suggest that there may be unique as well as shared disease pathways. The role of Class II MHC antigens is consistent with the idea that antigen is being presented to CD4 T cells under MHC control. The different MHC relationships suggest that the antigens may be different or at least different epitopes on the larger antigen. For the moment, PR3 and MPO are regarded as the important antigens in GPA and MPA respectively. In cases of EGPA with anti-MPO the same may apply [2].

<table>
<thead>
<tr>
<th>Risk factor/AAV</th>
<th>GPA</th>
<th>MPA</th>
<th>EGPA</th>
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<tr>
<td>MHC</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Y</td>
<td>Y</td>
<td>?</td>
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<tr>
<td>PTPN22</td>
<td>Y</td>
<td>Y</td>
<td>?</td>
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<tr>
<td>Antigen expression</td>
<td>Y-genetic</td>
<td>Y-epigenetic</td>
<td>?</td>
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<tr>
<td>SerpinA1</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>Silica</td>
<td>Y</td>
<td>Y</td>
<td>Y?</td>
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<tr>
<td>Low UVR/vitamin D</td>
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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 placental 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 SERPINA1 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


