Immunopathology of ANCA-Associated Vasculitis

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During the past few years remarkable progress has been achieved in the understanding of the pathogenic mechanisms leading to vascular inflammation and injury in ANCA-associated vasculitides (AAV): Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA) and Churg Strauss syndrome (CSS). In this paper we review the immunopathology of these diseases by describing the role of autoantibodies (ANCA), dysregulation and abnormalities at the cellular level, genetic background and environmental factors that predispose to autoimmune response.

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Key words: vasculitis, ANCA, WG, cytokine, T cells

Introduction

The vasculitides include a heterogeneous group of clinico-pathologic entities which share a common histopathologic finding: the inflammation within blood vessels resulting in vascular obstruction with tissue ischemia and infarction. Most of the vasculitic syndromes are mediated by immunopathogenic mechanisms which have been classified into four types (I–IV) of hypersensitivity reaction by Coombs and Gell. Accordingly, clinopathologic and immunohistochemical studies led to the terms allergic angiitis (type I reaction according to Coombs and Gell), ANCA-associated vasculitis (type II reaction), immune-complex vasculitis (type III reaction) and vasculitis associated with T cell mediated hypersensitivity (type IV reaction). Here we will concentrate on the ANCA-associated vasculitides.

ANCA-associated vasculitides include three related but clearly distinct disorders: WG, MPA and CSS. These entities share the characteristic of not having immune complex deposits in affected organs, and thus have been termed “pauci-immune”. Today, the diagnostic hallmarks of WG and MPA consist of a) clinical features (often pulmonary renal vasculitis syndrome), b) immunohistochemical studies (“pauci immune” vasculitis of the lung or glomerulonephritis (GN) with no or few immune deposits) and c) serological markers (PR3-ANCA or MPO-ANCA, respectively). Biopsies-usually taken from kidneys or lung-typically show a necrotizing vasculitis of the small arteries, capillaries or small veins. In the kidney, necrotizing GN with crescent formation is the most prominent feature; it cannot be distinguished from other forms of crescentic GN (e.g., Goodpasture’s syndrome) by light microscopy. However, immunohistochemistry shows no or only pauci-immune deposits in the ‘ANCA-associated vasculitides’ (WG, CSS, MPA), in contrast to Goodpasture’s syndrome (linear IgG deposits along the glomerular basement membrane) and Henoch-Schoenlein’s Purpura (HSP) (mesangial immunecomplexes of IgA) (1).

Unfortunately, our knowledge of the etiology and pathogenesis of AAV is still incomplete. We know that tissue destruction occurs because of host activity, but only a few of the triggers of this self-destructive process are actually known. The clue to the initiating event in AAV lies in environmental factors, such as infectious agents and environmental toxins, and genetic susceptibility. During the last few years, there is increasing evidence suggesting that ANCA play an active role in the immunopathogenesis of vasculitis. The pathogenesis of vasculitis includes different immunological mechanisms that induce in a final pathway of vascular inflammation that favours leukocytes to adhere to endothelial cells, penetrate into vessel walls, and release of injurious products.

In this review we will highlight the new immunogenetic aspects, environmental factors, the role of ANCA and the main cell populations (neutrophils (PMN) and T cells) and their products (i.e. enzymes, cytokines, etc.) relevant to the pathogenesis of AAV, especially of WG. The role and importance of these cells in the initiation and perpetuation of vasculitis and granuloma in these diseases will be discussed.

Environmental Factors

AAV are multifactorial diseases in which the relative contribution of each factor increases the relative risk of disease susceptibility. The etiology of AAV remains unknown. A combination of risk factors are involved in susceptibility to vasculitis. Genetic background may increase the risk but is insufficient to induce disease. It is hypothesized that environmental...
factors (i.e., infectious agents, drugs, chemical substances, etc.) are important modulating factors of most forms of vasculitis.

Several environmental agents have been suggested to be causally involved in the induction of vasculitis syndrome. In WG, a strong case has been made for *Staphylococcus aureus*, which may act through activation of leukocytes and the induction of an autoimmune response to neutrophil cytoplasmic antigens such as PR3 and MPO (2). Recently, we have examined the correlation between the nasal carriage of *Staph. aureus* in patients with WG and Ear-Nose-Throat (ENT) disease activity compared with RA patients. Our results suggest an association between nasal carriage of *Staph. aureus* and activity of WG in the upper respiratory tract (3).

Furthermore, Cohen-Tervaert et al, have currently discussed available evidence for a pathophysiological role of one possible environmental trigger, silica, in vasculitis. Exposure to silicon-containing compounds was found to be related to chronic renal failure or vasculitis. The mechanisms by which silica may induce ANCA-associated glomerulonephritis or vasculitis are not well known. Silicon-containing compounds have a pronounced adjuvant effect on immune responses, and silica particles are potent stimulators of lymphocytes and monocytes (4). Further, silica may induce apoptosis of monocytes and possibly neutrophils. Further epidemiologic studies are required to confirm this hypothesis.

The predominant involvement of the respiratory tract and the presence of neutrophilic alveolitis in patients with recent onset disease have led to suppose that inhaled agents may trigger the onset of WG. Recently, Duna et al have postulated that inhaled agents may stimulate the airways, thus triggering the disease in genetically or immunologically susceptible individuals. They assessed environmental exposures in a large cohort of patients with WG compared to various control populations. This study confirms the absence of seasonal differences in the onset of WG. It also demonstrates high rates of self-reported environmental exposures to inhaled substances in WG and all control populations. The absence of substantial differences in WG and controls may reflect the more important role of host susceptibility factors (5). More rigorous methods based on industrial hygiene assessment might further clarify the role of specific inhaled substances in the etiology of AAV.

Today, the triggers that can induce ANCA-positive vasculitis remain largely unknown. In a minority of cases, however, the disease appears to be medication-induced. There are many reports that treatment with hydralazine, or propylthiouracil is associated with AAV. A small number of case reports also implicate penicillamine, minocycline and allopurinol as agents capable of inducing an ANCA-positive vasculitis. The list of drugs implicated in ANCA-associated vasculitis is still growing and this potentially serious side effect of widely used medications call for future studies addressing the epidemiological and pathogenetic aspects of drug-associated vasculitis.

**Genetic components**

Little is known about the etiologic and genetic factors of the ANCA-associated diseases. In studies of families with an affected member, cases of siblings with WG have been reported (6). However, in our own series of 300 patients we only met 2 patients where another family member suffered from WG. Although WG has a racial bias, being predominantly a disease of Caucasians, the genetic factors involved are unknown (7).

Until recently, investigations of specific genes in WG have been mostly association studies directed at the MHC; this approach related to the known importance of MHC encoded molecules in the immune response and the fact that genetic analyses were facilitated by the highly polymorphic nature of genes within the MHC. The results of these studies are contradictory. Interestingly, a negative association was demonstrated between the presence of HLA-DR13D6 and the development of ANCA-associated vasculitis and glomerulonephritis (WG, MPA, idiopathic RPGN) (8).

Furthermore, it was suggested that free ANCA antigens (e.g. free proteinase 3, PR3) could mediate vasculitic lesions in the context of an acquired and/or genetically determined protease/antiprotease imbalance. A reproducible association has been found between ANCA activity and the deficiency alleles (Z and S) of alpha1-antitrypsin (α1AT) in ANCA-associated systemic vasculitis (9, 10). α1AT interacts with both of the major ANCA antigens: it is the main inhibitor of PMN’s neutral serine proteases (PR3 and HLE) and is inactivated by myeloperoxidase. The lack of PR3 inhibitor could induce an autoimmune response by exposure of free antigens (which are usually ‘hidden antigens’ in the azurophilic granules of the PMN or rapidly bound to α1AT after liberation) to the immune system. In addition, decreased neutralization of PR3 and HLE could cause increased damage at the site of inflammation with subsequently necrosis of the tissue (i.e. necrotizing granulomata and/or necrotizing arteritis). Furthermore, the interaction of PR3 with α1-AT can also inhibited by ANCA (11).

Recently, our group has investigated the polymorphism in the tumor necrosis factor (TNF) genes in WG, where the clinical activity could be correlated to the amount of TNFα production (12). The quantity of TNFα production is influenced by polymorphism of TNFα genes. Concerning TNFα the 2/2 phenotype of gene polymorphism at position 308 of the promoter region has been shown to produce higher amounts of TNFα compared with 1/1 and 1/2 phenotypes. Although the incidence of TNFα 2/2 phenotype was slightly elevated in WG (6% vs. 2% in healthy controls), no statistically significant differences could be demonstrated in TNFα gene polymorphisms including high and low producers of TNFα (13).

Furthermore, it has been demonstrated that a minor proportion of PR3 is expressed on the plasma membrane of normal circulating neutrophils in healthy individuals (14). The proportion of neutrophils carrying PR3 varied among individuals, but was highly stable for each person over time (15). Variability of the PR3 expression pattern is possibly controlled by genetic factors and individuals with a high proportion of membrane-associated PR3 may have an increased risk for WG (16). The discovery of a bi-allelic restriction fragment length polymorphism in the PR3 gene (17) and identification of a multi-
ANCA, PMN, Monocytes and Vasculitis

The serological hallmark of WG and MPA is the presence of circulating autoantibodies to either proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA). Some authors have also suggested that CSS is ANCA associated, but this remains controversial. Changes in titers of ANCA seem to reflect changes in disease activity. ANCA have been hypothesized to participate in the pathogenesis of necrotizing vasculitis based on their association with small vessel vasculitides and in vitro ability of these antibodies to activate neutrophils, monocytes and endothelial cells. Many of the basic features of ANCA response such as the factors responsible for the generation and perpetuation of these autoantibodies and the shaping of the ANCA immune response remain unknown.

Polymorphonuclear neutrophils (PMN) play an important role in the pathogenesis of WG: they are predominant at the site of tissue injury (necrotizing vasculitis and granuloma) and they are the main target cell for the ANCA's antigens. Using an HgCl₂-induced vasculitis model, recently direct evidence was provided for the primary role of PMN in vasculitis (20): PMN have been shown to be essential for induction of vasculitis and the degree of vasculitis correlated with the number of PMN.

Mononuclear phagocytes make up a significant proportion of the cell infiltrate in systemic necrotizing vasculitis lesions (21, 22). However, little is known about their role in vasculitis. Recently, Bruce et al, have demonstrated that monocytes from patients with primary systemic vasculitis (i.e., WG, CSS) have increased capacity to generate reactive oxygen species. Further study is required to determine if the reactive oxygen species generated act as a mechanism to promote vessel wall injury, or healing in this condition (23).

To explain, how the interaction of ANCA with their target antigens may result in necrotizing vasculitis, several mechanisms have been proposed. In inflammatory conditions, leukocytes have to migrate across the endothelial wall, which involves a complex process, to arrive at the site of inflammation.

This process is not accompanied by vessel wall injury (necrosis). However, in vasculitic conditions, if ANCA cause this vasculitis, the autoantibodies must interact with neutrophils and monocytes in the circulation, resulting in activation, microvascular adherence of leukocytes and subsequent vascular inflammation and necrosis (ANCA cytokine sequence theory). Clinical, pathological and experimental evidence of pathogenesis of vasculitis by ANCA are summarized in Table 1 and 2. One main conclusion from recent studies investigating the pathogenic potential role of ANCA, is that ANCA in combination with exogenous factors are able to aggravate a clinical inflammatory process and may result in systemic vasculitis and glomerulonephritis.

Experimental Models of ANCA-Associated Vasculitis and Glomerulonephritis

Several animal studies have been reported on ANCA and vasculitis/glomerulonephritis using various experimental approaches (24). There are few animal models for ANCA-related vasculitis that resemble microscopic polyangiitis. Kallenbergs group developed a model of proliferative GN resembling the renal involvement characteristic of MPA. The systemic autoimmune response to MPO led to severe necrotizing vasculitis with extracapillary GN and in some animals to extravascular granuloma formation. It is important to mention that induction of MPO-ANCA alone did not cause disease manifestations. Additional factors (infections?) inducing priming/activation of neutrophils, monocytes and endothelial cells with subsequently release of products (enzymes, oxygen species, etc.) are required. Similar models have been used to demonstrate PR3-ANCA-
Table 2. Experimental Evidence for the Pathogenic Role of ANCA

<table>
<thead>
<tr>
<th>Effect of ANCA on neutrophils</th>
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<tr>
<td>• priming and apoptosis of neutrophils results in cell membrane expression of the target antigens, making them accessible for ANCA</td>
</tr>
<tr>
<td>• cause degranulation and an oxidative burst of normal neutrophils primed with TNFα by binding simultaneously to FcγRII receptor and to the corresponding antigen expressed on the cell surface</td>
</tr>
<tr>
<td>• activation of neutrophils by ANCA involves 5-lipoxygenase pathway inducing the production of leukotriene (LTB4), a chemoattract for neutrophils</td>
</tr>
<tr>
<td>• induce expression interleukin-1β and IL-8 in neutrophils</td>
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<table>
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<tr>
<th>Effects of ANCA on monocytes</th>
</tr>
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<tbody>
<tr>
<td>• activate monocytes (induce ROI release and the production of monocyte chemoattractant-1, a potent for neutrophils; induce IL-8 production)</td>
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<tr>
<th>Effects of ANCA on endothelial cells (EC)</th>
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<tbody>
<tr>
<td>• induce expression of adhesion molecules and may enhance the adhesion of neutrophils and mononuclear cells to EC</td>
</tr>
<tr>
<td>• lysis of EC previously incubated with PR3 or MPO and neutrophils</td>
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</table>

<table>
<thead>
<tr>
<th>Effect of ANCA on PR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>• prevent inactivation of PR3 by natural inhibitor α1-antitrypsin</td>
</tr>
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</table>

An ANCA is caused by pathogenic antibodies against mouse PR3. ANCA-Associated vasculitis has not been developed. None of the experimental models completely mimics the human ANCA-associated diseases. Furthermore all of these in vivo animal models suggest but do not definitively prove, that ANCA are pathogenic.

**Wegener’s Granuloma: A Th1-Granulomatous Inflammation**

WG begins with granulomatous changes, as Fienberg was able to establish after decades of research. The primary granulomas form and develop in connective tissue, but without vascular involvement (30). In his last paper on this topic Friedrich Wegener wrote: “the vasculitis that accompanies the granulomatous disease is a secondary feature that represents a later stage” (31). Thus, insights into the leading immunopathogenic mechanisms in WG should come from studies concentrating on cells from the ‘pathergic granuloma’ or its surroundings (e.g. bronchoalveolar lavage: BAL).

Granuloma formation is usually a host tissue response to a foreign antigen. Studies of intercellular interactions that lead to formation and maintenance of granulomas have now focused on the role of T cells (32). In infectious diseases it has been found that in leprosy the T-cells locally produce the Th1-cytokine pattern, whereas the granuloma induced by the schistosome egg is an inflammatory reaction that is tightly controlled by Th2 cytokines (33, 34). Since the histological lesion of Wegener’s granuloma is largely composed of cells of the mononuclear cell lineage, the results suggest that a subset of MRL-lpr mice develops ANCA-related vasculitis rather than SLE and may be used as a spontaneous model for human MPA.

In another model, purified human PR3-ANCA injected into Balb/c mice caused production of mouse antibodies against human PR3-ANCA (Ab1), followed during the next month by production of anti-Ab1 antibodies (Ab2) that recognized human PR3. At the time of production of anti-Ab2s, the mice developed renal and pulmonary vasculitis similar to that in WG, suggesting a link between these two events. However, it is not clear from these experiments whether the antibodies (Ab2) also recognize murine PR3 (28). Recently, Jenne et al, have identified and characterized the murine PR3 (29). Despite the strong similarities between human and murine PR3, PR3-ANCA from WG patients did not recognize the natively folded murine PR3 indicating that endogenous murine homologue does not present any of those epitopes which are required to study human ANCA-induced vasculitis in mice. Consequently, it is unlikely that disease observed in mice after immunization with PR3-ANCA is caused by pathogenic antibodies against mouse PR3.

Until now, a satisfactory animal model for ANCA-associated vasculitis has not been developed. None of the experimental models completely mimics the human ANCA-associated diseases. Furthermore all of these in vivo animal models suggest but do not definitively prove, that ANCA are pathogenic.
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working hypothesis that WG could be a Th2-associated condition. On the other hand, studies on T cells from PB of WG patients have clearly shown that these cells exhibit increased secretion of IFN-γ but not of IL-4, IL-5 and IL-10, thus demonstrating that at least the periphery shows a Th1 response (37, 38).

Recently, our group has investigated the cytokine pattern (Th1 and Th2) in WG by analyzing the profile of cytokine secretion by T cells derived from tissue with granulomatous inflammation (nasal mucosal biopsy specimens) or from an area close to the site of granulomatous inflammation (BAL) and, for comparison, from PB. In this study we used different experimental approaches and IFN-γ (Th1 pattern) and IL-4 (Th2 pattern) were detected by ELISA and a competitive RT-PCR (“Table 3). Our results demonstrate that the Th1 pattern is the main cytokine profile exhibited by TCC isolated from nasal biopsy specimens displaying granulomatous inflammation and - to a lesser extent - by TCC and TCL generated from BAL cells. In addition, both polyclonal CD4+ and CD8+ T cells from PB and BAL produced predominantly IFN-γ. These findings fit well with the concept that T cells play a triggering role in the pathogenesis of WG.

WG and MPA are mainly differentiated by the occurrence of granulomatous inflammation in the former and the lack of granuloma in the latter (1). This difference resembles that between the 2 major forms of chronic inflammatory bowel disease, Crohn’s disease and ulcerative colitis. Several studies have shown enhanced IFN-γ and/or IL-2 mRNA expression, or increased IFN-γ protein production by T cells from the gut of patients with Crohn’s disease but not from patients with ulcerative colitis (39). In addition, surrogate marker studies have demonstrated increased sCD30 in ulcerative colitis but not in Crohn’s disease (40). Therefore, it was not entirely surprising that the few T-cell clones from our control patients with MPA did not produce Th1 cytokines.

Our results have demonstrated the existence of a clear-cut Th1 polarization of the immune response in the granulomatous inflammation in WG. Furthermore, very recently we analyzed the phenotype of inflammatory cells in nasal biopsies from patients with localized and generalized WG. The presence of CD26 (operational Th1 marker) and CD30 (Th2 marker) on T cells were detected by immunochemistry. Our findings indicate, that in nasal tissues mainly CD4+/CD26+ T cells as well as CD14+ monocytes/macrophages may contribute to a polarized Th1-like immune response in both phases of WG (22). The mechanisms responsible for the preferential development of Th1 cells in granuloma have not yet been investigated. Th1-dominant responses are very effective in eradicating infectious agents, including those hidden within the cells; however if the Th1 response is not effective or excessively prolonged, it may become dangerous for the host due to both the activity of cytotoxic cytokines and the strong activation of phagocytic cells. The local secretion of high levels of IFN-γ may represent an important amplification loop leading to a tissue-destructive inflammatory response in WG patients. IFN-γ activates local macrophages and granulocytes to produce pro-inflammatory cytokines and toxic metabolites, which cause damage to the tissue and maintain the inflammation.

As mentioned above, WG typically begins with a granulomatous inflammation in the respiratory tract without vasculitis and if untreated sometimes leads ultimately to ‘full-blown’ WG mainly as a consequence of the subsequent vasculitic disease. Considered in this manner, WG resembles other granulomatous diseases described above which are ultimately associated with the Th2 response. It remains to ask whether a Th1/Th2 switch occurs during the disease process since these 2 extremes of this disease spectrum are very difficult to detect. In light of ongoing advances in the development of immunotherapy modalities, it is imperative that these studies be performed in future.

**Conclusion**

The clinical picture and the pathogenesis of AAV (WG, MPA and CSS) is rather complex and involves a variety of immune mechanisms leading to necrotizing inflammation of blood

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Table 3. Th1/Th2 Cytokine Profiles in Wegener’s Granulomatosis

<table>
<thead>
<tr>
<th>Body compartment</th>
<th>Cells involved</th>
<th>WG</th>
<th>Disease controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal mucosa tissue</td>
<td>T/B cells; MØ; Eos.</td>
<td>Th1&gt;Th0</td>
<td>Chronic rhinitis: Th0</td>
</tr>
<tr>
<td>Tissue extracts</td>
<td>CD4+ T cells</td>
<td>Th1</td>
<td></td>
</tr>
<tr>
<td>Cloned T cells</td>
<td>CD4/CD8+ T cells</td>
<td>Th1&gt;Th0</td>
<td>MPA: Th2</td>
</tr>
<tr>
<td>BAL fluid</td>
<td>CD4+ T cells</td>
<td>Th1</td>
<td></td>
</tr>
<tr>
<td>CD3+ T cells</td>
<td>CD4/CD8+ T cells</td>
<td>Th1&gt;Th0</td>
<td></td>
</tr>
<tr>
<td>Cloned T cells</td>
<td>CD4+ T cells</td>
<td>Th1</td>
<td></td>
</tr>
<tr>
<td>T cell lines</td>
<td>CD4/CD8+ T cells</td>
<td>Th1</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>T/B cells; MØ</td>
<td>Th0&gt;Th1&gt;Th2</td>
<td>SLE Th0&gt;</td>
</tr>
<tr>
<td>MNC</td>
<td>CD4+ T cells</td>
<td>Th1&gt;Th0</td>
<td>SLE Th2&gt;</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>CD8+ T cells</td>
<td>Th1&gt;Th0</td>
<td>SLE Th2&gt;</td>
</tr>
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vessel walls. Although the events involved in this complex inflammatory process are partially known, the breakdown of immunological tolerance leading to PR3/MPO-ANCA remains unclear. Certain susceptibility genes and several environmental factors have been identified which probably influence the pathogenic mechanisms in these disorders. Moreover, in recent years, there has been considerable progress in recognizing the molecular and immunologic aspects of the effector mechanisms that mediate the process of vascular inflammation in AAV. In this regard, modulation of the inflammatory response by specific biologic response modifiers (i.e., cytokines, anti-cytokines, adhesions molecule antagonists, anti-Id ANCA, etc.) may play a pivotal role in the therapy of the AAV in the future.

References

11) Donohue et al. ELENNA et al
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