Pathogenesis of the vascular and glomerular damage in ANCA-positive vasculitis

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Introduction

Antineutrophil cytoplasmic autoantibodies (ANCA) are specific for antigens in the cytoplasm of neutrophils and monocytes. Approximately 90% of ANCA in patients with vasculitis or glomerulonephritis have specificity for either myeloperoxidase (MPO) or proteinase 3 (PR3), which are located in the primary granules of neutrophils and the peroxidase-positive lysosomes of monocytes.

Beginning with the initial publication about ANCA [1], they have been strongly associated with a spectrum of necrotizing small vessel vasculitides that includes Wegener’s granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome [2,3]. These vasculitides often have necrotizing and crescentic glomerulonephritis as a component of the systemic vascular injury. A pathologically identical glomerulonephritis also occurs as a renal-limited disease. There is mounting clinical, pathological, and experimental evidence that ANCA are pathogenic (Table 1).

The vasculitis and glomerulonephritis that are associated with ANCA are characterized, in the acute phase, by segmental fibrinoid necrosis of vessel walls, neutrophil and monocyte infiltration with leukocytoclasis, and the absence of immunohistological evidence for vascular wall localization of immune complexes or anti-glomerular basement membrane (anti-GBM) antibodies [4]. If ANCA cause this vasculitis, they must induce the following sequence of events: (i) leukocyte margination, adherence, and diapedesis; (ii) leukocyte activation with degranulation and generation of toxic oxygen metabolites; and (iii) vascular necrosis, resulting in karyorrhexis and fibrinous insudation. There are experimental data suggesting that ANCA can induce all of these events, at least in vitro [2,5].

Clinical evidence that ANCA are pathogenic

One of the earliest publications about the clinical characteristics of ANCA-vasculitis by van der Wouda et al. reported that ANCA were more frequent and at higher titer in patients with active Wegener’s granulomatosis compared to patients with quiescent disease [6]. Subsequently, there have been many publications that have addressed the correlation between ANCA titer and the clinical course of the disease [2]. Although there is controversy over whether or not the correlation is close enough to be used for making therapeutic decisions, there is general agreement that there is at least a rough positive correlation between ANCA-positivity and disease activity. This is consistent with a cause and effect relationship, but would also occur with an epiphenomenon that was secondary to active disease. In vitro experimental evidence: ANCA stimulate cytokine-primed neutrophils to degranulate and release toxic oxygen metabolites ANCA-activated neutrophils kill cultured endothelial cells ANCA-antigens adsorb to endothelial cells where they could participate in immune complex formation Endothelial cells may synthesize PR3

Table 1. Clinical, pathological and experimental evidence of pathogenesis of vasculitis by ANCA

Clinical and pathologic evidence:
- ANCA are very frequent in patients with necrotizing glomerulonephritis and vasculitis
- ANCA-disease responds to immunosuppressive treatment
- ANCA titers correlate with disease activity
- There is no evidence for anti-GBM or immune complex mediation of pauci-immune ANCA-vasculitis
- Drug-induced ANCA are associated with pauci-immune necrotizing vasculitis that disappears with discontinuation of the drug

In vivo experimental evidence:
- Animals with genetically determined or drug induced polyclonal B cell activation develop ANCA along with other autoantibodies, and develop glomerulonephritis and vasculitis
- Rats injected with subnephritogenic doses of anti-GBM in the presence of anti-MPO develop glomerulonephritis
- ANCA are induced in mice by an anti-idiotypic network response to human C-ANCA and develop pulmonary inflammation

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disease, as is the case with the erythrocyte sedimentation rate.

The dramatic response of severe acute ANCA-vasculitis to aggressive immunosuppressive therapy (e.g. with cyclophosphamide and high doses of corticosteroids) suggests some form of immune mediation, although this does not specifically incriminate the ANCA-immune response versus some other immune response. In addition, the immunosuppressive agents have nonspecific anti-inflammatory effects, such as depression of leukocyte production and function, and thus may cause disease remission by abrogating secondary mediator events rather than primary (aetiological) immunological events. The apparent effectiveness of pooled intravenous immune globulin in patients with ANCA-vasculitis is somewhat more supportive of an antibody-mediated pathogenic process [7].

The most compelling clinical evidence that ANCA cause vasculitis and glomerulonephritis is the observation that certain drugs, such as thiouracil, hydralazine, and penicillamine, are capable of inducing ANCA formation that is associated with the development of small vessel vasculitis and glomerulonephritis, which is pathologically identical to idiopathic pauci-immune ANCA-vasculitis and glomerulonephritis. This phenomenon has been described most often with propylthiouracil treatment [8–11]. For example, Tanemoto et al. [10] and D’Cruz et al. [11] have described five Grave’s disease patients who developed biopsy-proven pauci-immune crescentic glomerulonephritis after treatment with propylthiouracil. The disease resolved after withdrawal of the drug and immunosuppressive treatment.

**In vivo evidence that ANCA are pathogenic**

There are no experimental models that closely resemble pauci-immune ANCA-vasculitis or pauci-immune ANCA-glomerulonephritis, although there are an increasing number of models that appear to simulate certain ANCA-positive human disease states.

Approximately 20% of humans with systemic lupus erythematosus have ANCA, usually with specificity for MPO. Two strains of mice with polyclonal B cell activation and some pathological features that resemble lupus also have an ~20% frequency of ANCA, usually with specificity for MPO [12,13]. These mice develop vasculitis and glomerulonephritis, but the pathogenic role for ANCA is uncertain because only a minority of mice have ANCA and there are many other potentially pathogenic autoantibodies in addition to ANCA in these mice.

Mercury treatment of Brown Norway rats induces polyclonal B cell activation and the generation of multiple autoantibodies, including ANCA [14]. These animals develop vasculitis, especially in the gut. This model may be analogous to human drug-induced ANCA, which also is characterized by production of multiple autoantibodies. Once again, however, the presence of many different autoantibodies makes it difficult to incriminate any one as the specific cause of the vasculitis.

Brouwer et al. have developed a model of glomerulonephritis and vasculitis in rats that involves immunization of rats with human MPO [15]. The rats develop anti-MPO that crossreacts with rat MPO. Subsequent perfusion of the renal artery with a neutrophil granule extract and H2O2 causes crescentic glomerulonephritis. Control perfusion of kidneys in unimmunized rats does not induce comparable disease. Brouwer et al. observe transient immune complex formation in glomeruli between human MPO bound to vessel walls and anti-MPO. One explanation for this model is that the low level in situ immune complex formation acts as a nidus for amplification of inflammation by ANCA (i.e. anti-MPO); in other words, low level neutrophil activation initiated by the scanty immune complex formation is driven to severe acute inflammation by the presence of ANCA. This might be analogous to the development of severe crescentic glomerulonephritis in ANCA-positive humans who have a pattern of immune complex glomerulonephritides that usually would not induce severe inflammation. For example, patients with a membranous glomerulopathy pattern of immune complex deposition who have ANCA develop severe crescentic glomerulonephritis, possibly because the ANCA is driving the membranous glomerulopathy to a more inflammatory and necrotizing phenotype.

Kobayashi et al. reported observations that also suggest that ANCA can synergize with other pathogenic factors to cause disease [16]. They observed that rats injected with low doses of anti-GBM antibodies along with anti-MPO antibodies developed more severe glomerular neutrophil infiltration and fibrin formation than rats injected with anti-GBM or anti-MPO alone.

Recently, Heeringa et al. have reported results that extend the work of Kobayashi [17]. They immunized rats with human MPO, resulting in the production of circulating anti-MPO that reacted with human and rat MPO. Subsequent injection of subnephritogenic doses of anti-GBM antibodies caused crescentic glomerulonephritis in rats with circulating anti-MPO (i.e. ANCA), whereas rats that had not been immunized with MPO developed only insignificant glomerular lesions. This model further substantiates that ANCA can markedly amplify acute inflammatory events that are set into motion by stimuli that otherwise would cause only minor leukocyte activation.

Shoenfeld et al. have used a different strategy to induce ANCA in experimental animals [18,19]. They injected human ANCA with anti-PR3 specificity into the dermis of mice resulting in the formation of an anti-idiotypic antibody network. The anti-anti-idiotypic antibodies (Ab3) had anti-PR3 antigen specificity that mimicked the specificity of the human ANCA used for immunization. Although these mice developed minor pulmonary inflammatory lesions, the injury did not closely resemble ANCA-vasculitis.

Thus, there is no ideal animal model for pauci-immune ANCA-vasculitis in humans. The models of Brouwer and Heeringa [15,17] at least strongly suggest,
however, that ANCA can amplify acute inflammatory events once neutrophils are stimulated by low level proinflammatory events. This concept fits nicely with a number of in vitro observations that indicate the ability of ANCA to activate neutrophils that have been stimulated (primed) to express ANCA-antigens on their surfaces.

**In vitro evidence that ANCA are pathogenic**

Whatever the initiating event in patients with necrotizing vasculitis, there must be a final common pathway of injury that entails (i) leukocyte margination, adherence, and diapedesis, (ii) leukocyte activation with degranulation and generation of toxic oxygen metabolites, and (iii) vascular necrosis with fibrinous insudation. It is well accepted that localization of immune complexes or anti-basement membrane antibodies in vessel walls can initiate this final common pathway of vascular inflammation. Because most ANCA-positive vasculitis has no immunohistological evidence for either granular immune complex deposits or linear localization of anti-basement membrane antibodies, an alternative mechanism for initiating the pathway of vascular inflammation would seem likely. It is at least possible, however, that low levels of vessel wall immunoglobulin deposits, which are less than the detection sensitivity of immunohistology, are present in ANCA-positive vasculitis and are contributing to leukocyte activation.

The mechanisms for leukocyte activation by ANCA that have been proposed include: (i) direct activation of cytokine-primed neutrophils and monocytes by Fab2 binding to ANCA-antigens on leukocyte surfaces, (ii) Fc receptor engagement by ANCA-immune complexes in the leukocyte microenvironment, (iii) leukocyte interaction with immune complexes formed in situ in vessel walls between ANCA and ANCA-antigens released from leukocytes and adsorbed onto endothelial cells, and (iv) leukocyte interaction with immune complexes formed in situ in vessel walls between ANCA and ANCA-antigens produced by endothelial cells (e.g. PR3). There are in vitro experimental data that support each of these possibilities, which suggests that ANCA may initiate or augment inflammation by multiple synergistic mechanisms (Figure 1).

Any elucidation of a pathogenic mechanism that involves ANCA must explain how the autoantibodies are able to interact with antigens that are sequestered within the cytoplasm of neutrophils and monocytes. **In vitro** experiments have demonstrated that exposure of neutrophils to low doses of cytokines (e.g. tumour necrosis factor alpha, TNF) results in expression of small amounts of ANCA-antigens (e.g. MPO and PR3) at the cell surface, which can be detected by flow cytometry and immunoelectron microscopy [20,21]. If the cytokine dose is low enough, the neutrophils are primed to express ANCA-antigens but are not driven to full activation. Exposure of cytokine-primed neutrophils to ANCA IgG induces full activation with a respiratory burst that generates toxic oxygen metabolites, and degranulation that releases lytic and toxic enzymes [20–24]. The mechanism by which ANCA activate primed neutrophils is not completely understood. There is controversy over the relative roles of Fab2 binding versus Fc receptor engagement in the activation process. Immune complex formation between ANCA and ANCA-antigens would be expected on the surface of neutrophils and in the microenvironment immediately around primed neutrophils that are surrounded by the plasma or interstitial fluid of an ANCA-positive individual. This would result in engagement of neutrophil Fc receptors by the microenvironmental immune complexes. Such a scenario is supported by in vitro experiments that have indicated a role for Fc receptor engagement in the process of ANCA-induced neutrophil activation [22,23]. However, experiments by Kettritz et al. indicate that Fab2 (but not Fab) fragments of ANCA IgG are capable of activating cytokine-primed neutrophils in the absence of Fc receptor engagement [25]. Therefore, it appears that ANCA-induced neutrophil activation involves both specific recognition of surface antigens by the antigen-binding region of ANCA IgG, as well as engagement of Fc receptors by the Fc region of ANCA after binding to ANCA-antigens (Figure 1).

ANCA-induced activation of neutrophils in the presence of cultured endothelial cells results in endothelial cell death [26,27]. This process requires engagement of adhesion molecules by the neutrophils because the endothelial cell killing is inhibited by antibodies directed against the beta 2 integrin adhesion molecule family [28]. The endothelial cell killing probably involves both apoptosis and lytic necrosis. Until recently, neutrophil-induced cell death was thought to be a predominantly lytic necrotizing event. Although lytic necrosis certainly eventually occurs at sites of acute inflammation, apoptotic cell death may play an important role. This is supported by recent observations by Yang et al., which demonstrate that neutrophil granule enzymes, including PR3, induce apoptosis in cultured arterial endothelial cells [29].

Full activation of neutrophils and monocytes at sites of acute inflammation results in extensive release of highly charged ANCA-antigens that are capable of binding to cellular and matrix structures and thus acting as niduses for in situ immune complex formation with ANCA. Vargunam et al. have demonstrated in vitro that MPO binds to endothelial cells and thus is available to act as a planted antigen to interact with ANCA [30]. The Brouwer model of MPO-induced glomerulonephritis and vasculitis may involve a substantial component of in situ MPO-anti-MPO formation, at least during the early phases [15]. If in situ ANCA immune complex formation occurs in the tissues of patients with pauci-immune ANCA-vasculitis, it must be at very low levels because immunohistology usually demonstrates no IgG staining at sites of ANCA-vasculitis. Nevertheless, low levels of immune complexes may be present and may add fuel to the fire of ANCA-induced inflammation.
A very controversial issue pertaining to the pathogenic potential of ANCA is whether or not endothelial cells can actually synthesize and display ANCA antigens on their surfaces. If they can, ANCA could essentially act as anti-endothelial antibodies and bind directly to endothelial cells and kill them by an antibody-dependent cytotoxicity. Mayet et al. have detected PR3 in cytokine-stimulated human umbilical cord endothelial cells by immunohistochemistry and Western blot analysis, and have detected PR3 message in these cells by RT-PCR [31]. Mayet et al. have also made the in vitro observation that anti-PR3 has direct cytotoxic effects on endothelial cells [32]. In contrast, King et al. were unable to detect PR3 in cytokine-stimulated human umbilical cord endothelial cells by immunohistochemistry or enzyme immunoassay, and they were also unable to detect PR3 RNA message by PCR [33]. Thus, the importance of PR3 synthesis by endothelial cells is unresolved.

Several clinical observations suggest that some of the in vitro observations are paralleled by events in vivo. The onset and exacerbations of ANCA-vasculitis typically are preceded by flu-like symptoms, such as fever, myalgias and arthralgias, which suggest the presence of increased levels of circulating cytokines that could be priming neutrophils for interaction with ANCA. Such increased levels of circulating cytokines have been documented in patients with vasculitis [34–36], and circulating neutrophils in patients with ANCA-vasculitis have surface expression of ANCA-antigens [37].

**Hypothetical ANCA pathogenic mechanism**

In summary, the experimental data suggest that the activation of neutrophils (and by analogy, monocytes) by ANCA probably involves multiple synergistic events, including cytokine-induced expression of ANCA-antigens at the surface of the neutrophils, direct binding of ANCA via Fab'2 to antigens on the surface of neutrophils, engagement of Fc receptors by ANCA complexed with ANCA-antigens in the fluid microenvironment of the neutrophil and on vascular surfaces. At the bottom right, neutrophil activation ultimately results in endothelial cell and neutrophil apoptosis and necrosis, as well as lytic disruption of vessel wall matrix material.

Fig. 1. Diagram depicting hypothetical events in the induction of necrotizing vasculitis by ANCA. (This diagram depicts neutrophils, but monocytes would be activated in an identical fashion.) Beginning at the upper left, cytokine priming results in expression of ANCA-antigens at the neutrophil cell surface where they can interact with the Fab'2 of ANCA. Thereafter, neutrophils are activated by direct ANCA Fab'2 binding, as well as by engagement of Fc receptors by ANCA complexed with ANCA-antigens in the fluid microenvironment of the neutrophil and on vascular surfaces. At the bottom right, neutrophil activation ultimately results in endothelial cell and neutrophil apoptosis and necrosis, as well as lytic disruption of vessel wall matrix material.


