Whether anti-neutrophil cytoplasmic antibodies (ANCA) are pathogenic has been the subject of vigorous debate almost since their discovery. Before this question can be addressed more specifically, certain implications inherent to the question need to be clarified. First, what are ANCA and which ANCA are the focus of the question? Second, what is meant by “pathogenic” in this context? Different participants in the debate require different types of evidence before accepting an affirmative answer to the question. Some might insist on irrefutable evidence that ANCA cause or explain all clinical manifestations of the disease. Others might be satisfied with documentation of the pro-inflammatory effects of ANCA that are prerequisites for the development of certain specific disease manifestations (such as pauci-immune capillaritis or necrotizing glomerulonephritis). Yet others might accept a pathogenic role of ANCA if they can be shown to aggravate or modulate certain disease manifestations.

Immunoglobulin G (IgG) antibodies causing a cytoplasmic immunofluorescence pattern on ethanol-fixed human neutrophils detected in patients with clinical manifestations of a systemic small vessel vasculitis including necrotizing glomerulonephritis and with classic Wegener’s granulomatosis were first reported in the early 1980’s (1-3). These antibodies were descriptively called antineutrophil cytoplasmic antibodies (ANCA). Two different fluorescence patterns have been distinguished. The classic diffuse granular, centrally accentuated fluorescence pattern (C-ANCA) associated mostly with Wegener’s granulomatosis is caused by antibodies directed against proteinase 3 (PR3) (4). In contrast, a perinuclear fluorescence pattern (P-ANCA) can be caused by antibodies against a variety of neutrophil granule constituents (5).

P-ANCA directed against myeloperoxidase (MPO) were identified in most patients with microscopic polyangiitis, including its renal-limited variant (idiopathic pauci-immune glomerulonephritis), Churg-Strauss syndrome, and in a minority of patients diagnosed with Wegener’s granulomatosis (6-9). A large number of subsequent studies performed to evaluate the disease specificity and diagnostic utility of ANCA, paired with the identification of multiple potential target antigens for ANCA, have led to the following emerging themes. First, PR3-ANCA causing a C-ANCA fluorescence pattern and MPO-ANCA causing a P-ANCA fluorescence pattern have been firmly established as useful diagnostic adjuncts in the evaluation of patients suspected of having Wegener’s granulomatosis, microscopic polyangiitis, or Churg-Strauss syndrome. Because of this antibody association and the many shared clinical features of these diseases, they are increasingly referred to in cumulo as ANCA-associated vasculitis (AAV).

Second, ANCA directed against a variety of target antigens have been found in patients afflicted by viral, bacterial, mycobacterial or parasitic infections [reviewed in (5, 10, 11)]. In most of these patients, ANCA disappeared after successful antimicrobial therapy. This suggests that the primary ANCA response is a common, usually self-limiting result of cross-reactivity of antibodies formed against microbial epitopes bearing similarity to epitopes encountered on neutrophil granule constituents (epitope mimicry). In those patients who are predisposed to the development of autoimmune disease, tolerance for ANCA target antigens may be lost, and ANCA may persist beyond resolution of the infection. It has also been proposed that ANCA may be the result of idiotypic network activation triggered by an immune response di-
rected against antibodies of a primary immune response themselves, or against peptides with sequence stretches complimentary to those encountered on the autoantigen (12, 13)

Third, exposure to a variety of drugs can lead to the development of ANCA [reviewed in (14, 15)]. Many of the drugs are polyclonal B cell stimulating agents notorious for the development of autoimmune phenomena. Patients with drug-induced ANCA, particularly those with ANCA directed against MPO or PR3, seem to be prone to develop an AAV-like clinical syndrome.

Largely driven by the prominent clinical association of PR3- and MPO-ANCA with small vessel vasculitis, these types of ANCA have been the primary focus of investigation. Hence, this editorial aims (1) to provide an overview of the current evidence that supports a pathogenic role of these ANCA in AAV, (2) to point out limitations and inconsistencies of the evidence, and (3) to provide speculative explanations for perceived inconsistencies and open questions.

Clinical observations supporting a pathogenic role of PR3- and MPO-ANCA

The specific association of PR3-ANCA and MPO-ANCA with the ANCA-associated vasculitides is at the origin of any speculation about their potential pathogenicity. In contrast to organ-specific autoantibodies for which there is direct evidence of pathogenicity in diseases such as Graves’s disease, myasthenia gravis, or anti-glomerular basement membrane disease, the pathogenic role of autoantibodies in the systemic autoimmune diseases including systemic lupus erythematosus, anti-phospholipid syndrome and the small vessel vasculitides is not as readily apparent from clinical observations.

In Wegener’s granulomatosis and microscopic polyangiitis the presence of PR3- or MPO-ANCA seems to be related to the activity of small vessel vasculitis. Patients with biopsy-proven limited Wegener’s granulomatosis who remain ANCA-negative usually do not progress to generalized disease with small vessel vasculitic complications unless they develop ANCA during the course of their disease (personal observations). While ANCA may not reflect disease activity accurately in many patients (16, 17), prospective studies indicate that patients who turn ANCA-negative under therapy are unlikely to flare for as long as they remain ANCA-negative (18, 19). In fact, severe vasculitic flares without recurrence or persistence of ANCA are extremely rare in patients who were ANCA-positive at diagnosis, suggesting that ANCA are an essential requirement for the development of active small vessel vasculitides. However, as always in clinical medicine, there are occasional well-documented exceptions to the rule that challenge this concept.

The apparent efficacy of treatment modalities aimed at the removal of ANCA (plasma-exchange) or at the suppression of their production (B cell depletion) also seems to support their pathogenic role (20-22). Yet it has to be acknowledged that the effects of these interventions are much broader than the mere removal of these specific autoantibodies.

In vitro studies in support of a pathogenic role of PR3- and MPO-ANCA

Many elegant in vitro studies have documented a variety of pro-inflammatory effects of ANCA that cumulatively lead to small vessel endothelial cell and tissue damage [reviewed in detail in (23-25)]. ANCA may increase the adhesion of neutrophils to endothelial cells by enhancing the expression of cell adhesion molecules on endothelial cells. ANCA seem to contribute to tissue damage by activating primed neutrophils, which results in the release of oxygen radicals and proteolytic enzymes. The latter may in turn induce endothelial cell apoptosis. ANCA-mediated neutrophil activation involves both Fcγ-receptor engagement and recognition of expressed target antigen on the surface of primed neutrophils. ANCA may also cause endothelial cell damage by direct antibody-dependent cellular cytotoxicity or by localized immune complex formation with target antigens bound to the endothelial cell surface which may initiate localized complement activation. Finally, ANCA may contribute to the recruitment of more inflammatory cells to the area of tissue injury by stimulating the release of chemotactic chemokines and agents from neutrophils, monocytes, and endothelial cells. The resulting shift in the chemotactic gradient could lead to trapping of the activated inflammatory cells at the endothelial cell interface or within the wall of small vessels.

Most ANCA-mediated effects on neutrophils and monocytes observed in vitro require priming of the cells with tumor necrosis factor-α (TNF-α). In vivo, cytokine-dependent priming of inflammatory cells is not unique to vasculitis. Cytokine stimulation of neutrophils and monocytes, typically by TNF, occurs normally in the context of every infection, and results in the increased surface expression of ANCA target antigens. Patients with active vasculitis have indeed been shown to have both elevated levels of TNF and increased expression of ANCA target antigens on the surface of their neutrophils (26-32). In combination, these observations allow the hypothesis that the priming of neutrophils and monocytes occurring in response to cytokine stimulation during infection enables ANCA to interact with their target antigens on neutrophil or monocyte surfaces. This in turn sets the documented pro-inflammatory effects of ANCA in motion, which aggravate and perpetuate the inflammatory reaction at the endothelial cell interface.

This concept is consistent with clinical observations in which the onset or recurrence of disease activity is frequently linked to preceding infections or other potential inflammatory stimuli. On the other hand, it is also consistent with the fact that many patients with persistent or recurrent ANCA do not immediately suffer a flare of their disease (18, 33). However, the concept of ANCA exerting pathogenic effects by activating neutrophils in the blood via their interaction with the target antigen expressed on the neutrophil surface has recently been challenged by experi-
ments suggesting that PR3-ANCA from Wegener’s patients are low affinity antibodies (34). Binding of PR3-ANCA IgG to primed neutrophils was only detectable in a high concentration/low reaction volume setting, and was inhibited by plasma. This is in clear contrast to heterologous polyclonal or monoclonal anti-PR3 or anti-MPO antibodies or autologous polyclonal antibodies raised in knock-out mice, all of which seem to be high affinity antibodies (34). While the observations suggesting a low affinity of PR3-ANCA from WG patients for neutrophil surface PR3 seem to challenge the ANCA-neutrophil activation theory, they do not exclude the possibility of activated neutrophils coming into contact with high concentrations of ANCA at the site of inflammation and particularly in capillaries. The high concentration/low reaction volume conditions under which binding of PR3-ANCA to neutrophil surfaces could be demonstrated (34), are most likely to be given in the lumen of capillaries, but not large vessels.

ANCA may also contribute to the development of tissue necrosis by dysregulating the clearance of apoptotic neutrophils by macrophages (35). ANCA seem to accelerate the apoptosis of primed neutrophils, as evidenced by the morphologic nuclear changes of apoptosis (35). However, the same study failed to detect an increase in plasma membrane expression of phosphatidyserine on ANCA-exposed neutrophils undergoing accelerated apoptosis (35). Phosphatidyserine is an annexin V binding site which promotes the reneutrophil apoptosis have also been proposed (37,38). As ANCA target antigens are exposed on the surface of apoptotic neutrophils (39,40), ANCA can opsonize them. The opsonization of apoptotic neutrophils by ANCA seems to accelerate their phagocytosis by macrophages, and this process is associated with increased release of the proinflammatory cytokines, interleukin 1, interleukin 8 and tumor necrosis factor-α from macrophages (37,38). Whether and how these mechanisms documented in vitro with monoclonal anti-PR3 and anti-MPO antibodies or affinity purified ANCA-IgG preparations unfold in vivo remains speculative.

Animal models supporting a pathogenic role of ANCA

As the clinical and in vitro experimental evidence for a pathogenic role of ANCA in ANCA-associated vasculitis has remained indirect, investigators have turned to animal models for more direct evidence. To date most insights have been derived from MPO-ANCA models. Several studies performed in rodent strains prone to develop autoimmunity and in rodents exposed to T-cell-dependent polyclonal B cell stimulation (mercuric chloride treatment) have indicated that the presence of MPO-ANCA modulates the disease severity and phenotype, favoring the development of vasculitis (41, 42). In addition, they have shown that the disease phenotype is shaped by the genetic background and by environmental factors such as infections (42-44). The specific role of MPO-ANCA is somewhat difficult to interpret in these models because a variety of other autoantibodies and abnormalities of the immune response coexist.

Studies designed to more specifically address the pathogenic role of MPO-ANCA as a single variable were performed in Brown-Norway rats. When immunized with human MPO, these animals develop antibodies that cross-react with rat MPO. It seems that these antibodies alone do not cause tissue injury or vasculitis in the Brown-Norway rat, yet vasculitis develops in their presence provided they can interact with their target antigen (and its substrate). Furthermore, the location of the developing pathology is dependent on the location of this interaction between the antibodies and target antigen (45-47). Whether this is truly a model for pauci-immune vasculitis as originally suggested (45), or rather of immune complex-mediated disease (48), remains unsettled. Using this and other models, it has also been shown that anti-MPO antibodies aggravate tissue lesions resulting from other mechanisms of injury (49-51).

To date, the most compelling evidence for the pathogenicity of MPO-ANCA comes from a series of experiments based on the generation of anti-MPO antibodies in MPO-deficient mice immunized with murine MPO purified from a murine myeloid cell line (52). In these experiments, both the transfer of spleen cells and IgG preparations into recombinase activating gene (Rag2)-deficient mice or wild type mice caused lesions reminiscent of pauci-immune necrotizing glomerulonephritis and vasculitis in other organs including the lung. In contrast, recipients of the transfer of splenocytes or IgG preparations from MPO-deficient mice immunized with bovine serum albumin did not develop significant pathology. Nor did pathology develop when splenocytes or IgG from MPO-immunized animals were transferred into MPO-deficient mice. These experiments not only confirm that MPO-ANCA are pathogenic in vivo and that the interaction between MPO-ANCA and the target antigen is a requirement for developing pathology, they are also the first to suggest that MPO-ANCA alone can induce tissue injury in the absence of other factors.

Efforts to create a model for PR3-ANCA-associated vasculitis have met with many more challenges. Most of these seem to originate from substantial structural and functional differences between human and murine PR3. As a consequence, the immunization of rodents with human PR3 has not resulted in antibodies cross-reacting with the rodent PR3. This prompted the immunization of PR3-deficient mice with recombinant murine PR3 (53). The resulting murine anti-PR3 antibodies bind to its target antigen expressed on
the surface of activated neutrophils from wild type mice (53). The transfer of murine PR3-ANCA containing sera, but not of control sera from sham-immunized animals into wild-type mice had a significant pro-inflammatory effect; in the presence of murine PR3-ANCA a significant augmentation of TNF-α induced skin inflammation occurred. This study is the first to date to document a pathogenic effect of species-specific PR3-ANCA in vivo (53). In contrast to the MPO-ANCA vasculitis mouse model, the presence of these murine PR3-ANCA alone did not seem to cause any pathologic effect. This observation is consistent with clinical observations indicating that ANCA alone may not be sufficient to cause disease, but that an inflammatory environment or triggering injury is required for ANCA to exert their pro-inflammatory pathogenic effects. However, it remains unclear why the mice exposed to inflammatory stimuli in the presence of murine PR3-ANCA did not develop typical vasculitis lesions (53). Taken together, the various animal models have provided substantial support for a pathogenic role of ANCA in vivo, ranging from the documentation of pro-inflammatory, disease-modifying and injury-enhancing effects to the direct induction of pauci-immune glomerulonephritis lesions. Nevertheless, the artificial experimental conditions of animal models and the species differences preclude uncritical extrapolations to the human condition.

Not all ANCA are equally pathogenic
If one accepts that ANCA exert pathogenic effects in ANCA-associated vasculitis by various and quite different pro-inflammatory mechanisms, the heterogeneous clinical manifestations and the at times contradictory results of in vitro experimentation are best reconciled by conceding that different ANCA subsets have different pathogenic potential. As postulated, pathogenic effects of ANCA require molecular interactions of ANCA with their target antigens, and therefore it is not surprising that different ANCA are associated with different disease manifestations and outcomes. Rare exceptions aside, only patients with PR3- and MPO-ANCA seem to develop small vessel vasculitis, but not those with ANCA directed primarily against other target antigens. Among patients with a diagnosis of Wegener’s granulomatosis or microscopic polyangiitis, the presence of PR3-ANCA portends a higher mortality, higher relapse rate, and risk of more rapid loss of renal function in comparison to MPO-ANCA (54-57). In addition, a variety of different PR3-ANCA and MPO-ANCA subsets have been documented in small patient populations. Such subsets include ANCA of different isotypes (58), or ANCA recognizing preferentially the pro-form conformation or specific epitopes of their target antigens (59-62). In individual patients, such ANCA subsets may change in a variable fashion over time (59, 62). Available routine methods of ANCA detection do not distinguish these different ANCA subsets. Whether specific ANCA subsets can explain why some patients with persistent ANCA or ANCA titer increases relapse promptly, whereas others don’t remain to be validated by determining their clinical and functional significance in large longitudinal patient cohorts.

In conclusion, evidence supporting a pathogenic role of ANCA continues to accumulate. Yet, the evidence derived from clinical observations and detailed in vitro studies remains circumstantial. The most recent evidence from animal models is very compelling, yet not absolutely beyond any doubt. The debate about the pathogenicity of ANCA is likely to continue (63) until we have the tools to selectively remove ANCA (and only ANCA) from a patient with active ANCA-associated vasculitis, and see a therapeutic benefit in the patient after ANCA removal as the sole therapeutic intervention.

References
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