Pathogenesis of Lung Vasculitis

Patrick H. Nachman, M.D.¹ and Ashley G. Henderson, M.D.²

ABSTRACT

Vasculitides that affect the lung represent a diverse group of diseases with various systemic clinical manifestations, and include microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA, formerly Wegener granulomatosis), Churg-Strauss syndrome (CSS), and anti-glomerular basement membrane (anti-GBM) disease (Goodpasture syndrome). The etiologies of these diseases remain largely unknown. Although the pathogenic mechanisms of each differ, these diseases overlap by the presence of anti-neutrophil cytoplasmic autoantibodies (ANCAs) in the vast majority of patients with MPA and GPA, and a substantial minority of patients with CSS and anti-GBM disease. This article reviews the current understanding of the pathogenesis of these four disease entities.

KEYWORDS: Vasculitis, microscopic polyangiitis, Wegener granulomatosis, Churg Strauss syndrome, Goodpasture syndrome, anti-GBM, pathogenesis

ANCA-ASSOCIATED SMALL-VEssel VASCULITIS

Pathogenesis

The pathogenesis of small-vessel-vasculitis associated with the presence of ANCA is not fully understood.5,6 There is convincing evidence that ANCAs are directly involved in the pathogenesis of pauci-immune small-vessel vasculitis or glomerulonephritis based on substantial in vitro and in vivo data. In vitro, ANCAs have been shown to activate normal human polymorphonuclear leukocytes.7–9 The current hypothesis stipulates that ANCAs induce a premature neutrophil respiratory burst and degranulation of primary and secondary granules10,11

¹University of North Carolina Kidney Center, ²Division of Pulmonary and Critical Care Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina.

Address for correspondence and reprint requests: Patrick H. Nachman, M.D., UNC Kidney Center, Campus Box 7155, University of North Carolina, Chapel Hill, NC 27599 (e-mail: patrick_nachman@med.unc.edu).

Lung Vasculitis and Alveolar Hemorrhage; Guest Editor, Loic Guillemin, M.D.
Semin Respir Crit Care Med 2011;32:245–253. Copyright © 2011 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.
ISSN 1069-3424.
at the time of their margination and diapedesis, leading to the release of lytic enzymes and toxic oxygen metabolites at the site of the vessel wall, thus leading to endothelial cell damage \(^{12,13}\) and necrotizing inflammatory injury. In order for anti-myeloperoxidase (anti-MPO), or anti-proteinase-3 (anti-PR3), to interact with their corresponding antigens, they must penetrate the cell; alternatively, the antigens must translocate to the cell surface. Indeed, small amounts of cytokines [eg, tumor necrosis factor (TNF) and interleukin-1 (IL-1)] at concentrations too low to cause full neutrophil activation, induce the translocation of MPO and PR3 to the cell surface.\(^{14,15}\) Furthermore, patients with ANCA disease aberrantly express PR3 and MPO genes through epigenetic mechanisms, and this expression correlates with disease activity.\(^{16,17}\)

In addition, PR3 and MPO released from neutrophils and monocytes enter endothelial cells and cause cell damage. PR3 entry into the endothelial cells\(^{18,19}\) results in the production of IL-8\(^20\) and chemotactant protein-1 and induces an apoptotic event.\(^{21,22}\) MPO can transcytose intact endothelium to localize within the extracellular matrix\(^{23}\) where it catalyzes nitration of tyrosine residues on extracellular matrix proteins,\(^{24}\) resulting in their fragmentation.\(^{25}\) Recent data suggest that endothelial cells inhibit superoxide generation by ANCA-activated neutrophils, and that endothelial cell injury may be mediated by serine proteases instead.\(^{26}\)

Neutrophil activation by ANCAs is likely mediated by both the antigen-binding portion of the auto-antibodies [F(ab')\(2\)] and the engagement of their Fc fraction to Fc gamma receptors on the surface of neutrophils. Engagement of the Fc receptors results in neutrophil activation including respiratory burst, degranulation, phagocytosis, cytokine production, and upregulation of adhesion molecules.\(^{13,27}\) ANCAs have been shown to engage both Fc gamma RIIa and Fc gamma RIIib receptors.\(^{28-30}\) In addition, ANCAs F(ab')\(2\) induce oxygen radical production\(^{28}\) and the transcription of cytokine genes in normal human neutrophils and monocytes.\(^{31}\) Overall, ANCAs F(ab')\(2\) are likely capable of low-level neutrophil and monocyte activation, whereas the Fc portion causes leukocyte activation once the F(ab')\(2\) has interacted with the antigen, either on the cell surface or in the microenvironment.\(^{13}\)

The role of T cells in the pathogenesis of pauci-immune necrotizing small-vessel vasculitis is suggested by the presence of CD4+ T cells in the granulomatous and active vasculitic lesions,\(^{32-35}\) and by correlation of soluble markers of T cell activation with disease activity, specifically soluble interleukin-2 receptor\(^{36}\) and sCD30.\(^{37}\)

A substantial body of work examined T cell abnormalities in ANCA disease, primarily in Wegener granulomatosis.\(^{38}\) Studies indicate there can be generalized leukopenia with low numbers of CD4+ T helper cells independent of therapy, increased effector memory T cells in remission, a skewing toward more PR3-responsive Th17 cells,\(^{39}\) and perhaps a functional defect in regulatory T (Treg) cells whereby these cells were found to have diminished or absent suppression of autologous responder T cell proliferation in co-incubation studies.\(^{40}\) In addition, there is an increased percentage of T cells secreting IL-17 in the periphery and serum levels of TH17-associated cytokine IL-23 correlate with the propensity for disease activity.\(^{41}\)

Recent animal models have provided direct evidence of the pathogenic role of ANCAs in small-vessel vasculitis and helped elucidate involved inflammatory pathways. Rats immunized with human MPO develop anti-rat-MPO antibodies, a necrotizing and crescentic glomerulonephritis, and pulmonary capillaritis.\(^{42}\) Using intravital microscopy, elegant studies have shown that anti-MPO-activated neutrophils marginate and diapedes along the vascular wall.\(^{42}\) In another model, MPO knockout mice were immunized with murine MPO, and splenocytes from these mice were transferred to immune-incompetent Rag2 mice resulting in the development of anti-MPO antibodies, a severe necrotizing and crescentic glomerulonephritis, and, in some animals, vasculitis in the lung and other organs. The transfer of anti-MPO antibodies alone induces a pauci-immune necrotizing and crescentic glomerulonephritis thus establishing the ability of anti-MPO antibodies to induce disease.\(^{43,44}\) The disease is abrogated when the neutrophils of anti-MPO recipient mice are depleted by a selective anti-neutrophil monoclonal antibody thus indicating the important role of neutrophil in the pathogenesis of this disease.\(^{45}\) Conversely, the transfer of T cells alone did not cause glomerular crescent formation or vascular necrosis.\(^{46}\) Using this same model, a previously unsuspected role of complement activation was demonstrated. Glomerulonephritis and vasculitis were abrogated with cobra venom factor and failed to develop in mice deficient for complement factors C5 and B, whereas C4-deficient mice developed disease comparable with wild-type mice.\(^{47}\) These results indicate that the alternative complement pathway (but not the classic or lectin pathways) is required for disease induction. Furthermore, the glomerulonephritis is completely abrogated or markedly ameliorated by treating the mice with a C5-inhibiting monoclonal antibody.\(^{48}\) These results are corroborated by in vitro experiments that demonstrate that blockade of the C5a receptor on human neutrophils abrogated their stimulation.\(^{49}\) In aggregate, these results suggest an important role of complement activation in the pathogenesis of ANCA vasculitis; however, their relevance to disease in humans remains to be established.

Animal models establishing the pathogenic role of anti-PR3 are less well established. In a recent study, autoimmunity-prone non-obese diabetic (NOD) mice were immunized with recombinant mouse PR3 that
Induction of ANCA

As is true for most autoimmune responses, the inciting events in the loss of tolerance and the generation of ANCA are not known. Although genetic predispositions and environmental exposure have been implicated, no direct link between these exposures and the formation of ANCA has been established. Three hypotheses have been advanced possibly leading to the generation of ANCA.

A serendipitous finding in ANCA vasculitis led to a theory of autoantigen complementarity. This theory rests on evidence that proteins transcribed and translated from the sense strand of DNA bind to proteins that are transcribed and translated from the anti-sense strand of DNA. It has been demonstrated that some patients with PR3-ANCA harbor antibodies to an antigen complementary to the middle portion of PR3. These anti-complementary PR3 antibodies form an anti-idiotypic pair with PR3-ANCA. Moreover, cloned complementary PR3 proteins bind to PR3 and function as a serine proteinase inhibitor. Preliminary data suggest that the complementary PR3 antigens are found on a variety of microbes, some of which have been associated with ANCA vasculitis and also found in the genome of some patients with both PR3- and MPO-ANCA.

Although these studies need to be confirmed and expanded to determine the source of the complementary PR3 antigen and their role (if any) in inducing vasculitis, they provide a novel venue for understanding the proximate cause of the ANCA autoimmune response.

A second theory for the genesis of ANCA disease is based on the observation that some patients with both MPO-ANCA and PR3-ANCA have antibodies to another neutrophil protein, lysosome-associated membrane protein 2 (LAMP2), that are capable of neutrophil activation and endothelial damage in vitro. LAMP2 has homology to a protein expressed by fimbriated bacteria (FimH). Antibodies to either FimH peptides or LAMP2 peptides are capable of inducing necrotizing and crescentic glomerulonephritis in rats. Anti-LAMP2 antibodies could therefore result from molecular mimicry as a result of infection with gram-negative organisms making FimH. The association of LAMP2 antibodies with ANCA disease has not yet been independently confirmed.

A third proposed theory of causation rests on the concept of so-called neutrophil extracellular traps (NETs), consisting of chromatin fibers released by ANCA-stimulated neutrophils, and containing the ANCA antigens MPO and PR3, as well as a neutrophil-derived activator of plasmacytoid dendritic cells. Although not specifically implicated in inducing ANCA, this paradigm proposes a mechanism by which the formation of NETs could activate dendritic cells and autoreactive B cells, capable of perpetuating the autoimmune response to the ANCA target antigens.

Churg-Strauss Syndrome

Churg-Strauss syndrome (CSS) is characterized by the presence of eosinophil-rich granulomatous inflammation involving the respiratory tract, and a necrotizing vasculitis involving primarily the small and medium-size vessels. It is clinically characterized by the presence of asthma and peripheral blood eosinophilia and allergic rhinitis. Only ~40% of patients have circulating ANCA, which, when present, are usually directed against MPO (MPO-ANCA). ANCA-positive and ANCA-negative CSS appear to differ to some extent with regard to the frequency and character of organ involvement. ANCA-positive patients are more likely to manifest signs of necrotizing glomerulonephritis, pulmonary hemorrhage, peripheral neuropathy, and purpura, whereas ANCA-negative patients are more likely to present cardiac involvement and pulmonary infiltrates.
Upon careful review of all cases of suspected drug-induced CSS reported to the FDA between 1996 and 2003 (n = 1274), the diagnosis could be confirmed in 181 cases, 90% of whom had a preceding exposure to LTRA. A positive ANCA test was detected in 42% of the cases tested. Of 140 cases for whom there was sufficient documentation to categorize the patterns of disease presentation and corticosteroid use, 9% had a previous diagnosis of CSS, corticosteroids were decreased or stopped within 6 months in 19%, 8% were possibly in a prodromal early phase of CSS at the time the LTRA was initiated, 20% had unstable asthma at the time of treatment initiation, and 44% had stable asthma at treatment initiation. The latter two categories suggest that the use of LTRA may be causative of CSS in some patients.

**PATHOGENESIS**

Understanding the pathogenesis of CSS has focused on mechanisms involved in the development of the eosinophilia characterizing this disorder, and notably a key role for IL-5 and other Th2 cytokines. Patients with active CSS appear to have elevated plasma levels of IL-5, and peripheral blood mononuclear cell production of IL-5 may be increased in vitro in the setting of T cell activation. IL5 induces terminal differentiation of committed eosinophil precursors, prolongs their survival, and induces their degranulation and antibody-dependent cytotoxicity, and promotes their adhesion to endothelial cells and transmigration from the vasculature. In addition to IL-5, T cells from patients with CSS exhibit increased production of Th2 cytokines IL-4 and IL-13. Migration of eosinophils to inflammatory sites appears to be mediated by eotaxin-3, and increased levels of this cytokine appear to be correlated with levels of disease activity and inflammation.

Peripheral T cell lines from CSS patients also produce high amounts of the Th-1 cytokine gamma-interferon (IFNγ). One study revealed that patients with CSS exhibit decreased CD4 + CD25 + T cells that produce IL-10 when compared with chronic eosinophilic pneumonia and asthma, which increased in patients with CSS in remission. These findings again suggest a role of T regulatory cells in the development of the disease. It is conceivable that the relative importance of Th-1 and Th-2 responses may differ in patients with predominantly eosinophilic/allergic phenotype versus vasculitic/granulomatous manifestations of the disease. In addition, patients with active CSS exhibit decreased frequency of peripheral Treg cells and increased frequency of Th17 cells when compared with patients with asthma and chronic eosinophilic pneumonia. These findings were reversed in patients with inactive disease.

Genetic predisposition to the development of CSS has been proposed. In one study, HLA (human leukocyte antigens) -DRB4 and HLA-DRB1*07 were more prevalent among patients with CSS, with HLA-DRB4 correlating with the number of vasculitic manifestations. In another study, three single nucleotide polymorphisms tagging a haplotype of the IL-10 gene promoter that were found to be highly significantly associated with ANCA-negative CSS (n = 103) (odds ratio 2.16, 95% confidence interval 1.52–3.06), but were negatively associated with Wegener granulomatosis (n = 403).

**Goodpasture Syndrome (Anti-glomerular Basement Membrane–Associated Lung Vasculitis)**

Goodpasture syndrome is characterized by circulating antibodies to the GBM (anti-GBM) and deposition of immunoglobulin G (IgG) or rarely IgA along GBMs. Anti-GBM disease occurs as a renal–limited disease (anti-GBM glomerulonephritis) and as a pulmonary–renal vasculitic syndrome (Goodpasture syndrome). The incidence of anti-GBM disease has two peaks with respect to age. The first peak is in the second and third decade of life and has a male preponderance and a higher frequency of pulmonary hemorrhage (Goodpasture syndrome). The second peak is in the sixth and seventh decade and has a predominance of women who more often have renal-limited disease.

Genetic susceptibility to anti-GBM disease is associated with HLA DRB1*1501 and DQB*0602, supporting the hypothesis that certain class II molecules present GBM peptides to T helper cells. This concept is further supported by mouse models of anti-GBM disease in which crescentic glomerulonephritis and lung hemorrhage are restricted to only certain MHC (major histocompatibility complex) haplotypes, despite the ability of mice of all haplotypes to produce antibodies to the noncollagenous (NC1) domain of the α3 chain of type IV collagen. In addition to genetic susceptibility based on HLA antigens, differences in susceptibility to anti-GBM disease in mouse strains were associated with haplotypes of the kallikrein gene family, which encodes serine esterases implicated in the regulation of inflammation, apoptosis, redox balance, and fibrosis. Antagonizing the kallikrein pathway by blocking the bradykinin receptors augmented disease, while bradykinin administration reduced the severity of anti-GBM antibody-induced nephritis in a susceptible mouse strain. These results suggest that kallikreins are protective disease–associated genes in anti-GBM antibody-induced nephritis in mice. Similarly, study of various strains of rats to nephrotoxic serum (rabbit anti-rat GBM serum) has identified the duplication of the Fc gamma R3 gene as a determinant of resistance to disease. Conversely, the susceptibility of Wistar Kyoto (WKY) rats has been linked to a polymorphism of the JunD gene, associated
with regulation of macrophage activation. Whether these findings pertain to susceptibility or severity of anti-GBM disease in humans is unknown.

PATHOGENESIS
The landmark study by Lerner et al. demonstrated that antibodies eluted from kidneys of patients with Goodpasture syndrome and injected in monkeys led to the induction of fulminant glomerulonephritis, proteinuria, renal failure, and pulmonary hemorrhage along with intense staining of the GBM for human IgG.

The antigen to which anti-GBM antibodies react is the collagenase-resistant part of type IV collagen, the “noncollagenous domain,” or NC1 domain. The antigenic epitopes are in a cryptic form because antibody binding to the native hexameric structure of the NC1 domain is minimal but increases 15-fold when the hexameric NC1 domain is denatured and dissociates into dimers and monomers. About 90% of anti-type IV collagen antibodies are directed against the α-3 chain of type IV collagen, sequestered within the α3εα5(IV) NC1 hexamers. In patients with anti-GBM disease who do not have antibodies to the classic epitopes on the α 3 chain, antibodies to entactin 

The majority of patients with anti-GBM disease express antibodies to two major conformational epitopes (EA: residues 17–31 and EB: residues 127–141) located within the carboxyterminal NC1 domain of the α-3 chain of type IV collagen. Antibody levels against α3, EA, and EB correlated with serum creatinine and with death or end-stage renal disease (ESRD) at 1 year, but not with sex, age, presence of ANCAs, or hemoptysis. The stimuli leading to the formation of anti-GBM autoantibodies are unknown; is the mechanism by which the normally hidden target epitopes become accessible to circulating autoantibodies. It is presumed that environmental factors, such as exposure to hydrocarbons, tobacco smoke, and endogenous oxidants can expose the cryptic Goodpasture epitopes.

About one third of patients with anti-GBM/Goodpasture disease also have circulating ANCA, the majority being to MPO. No differences in the reactivity to the major Goodpasture epitopes were detected between patients with anti-GBM plus ANCAs compared with anti-GBM alone. It is speculated that in patients with concomitant anti-GBM and ANCAs, the latter may appear first and cause damage to the GBM, thus exposing the normally hidden target epitopes of anti-GBM antibodies. Coexistence of ANCAs in patients with anti-GBM antibodies is associated with small-vessel vasculitis in organs in addition to lung and kidney. In experimental models, antibodies to MPO aggravate experimental anti-GBM disease.

Several animal models of anti-GBM disease were developed based on immunization with heterologous or homologous GBM. Anti-GBM antibody–induced injury can also be induced passively by the intravenous injection of heterologous anti-GBM antibodies. The rat model induced by injection of heterologous anti-GBM has permitted the study of the roles of various inflammatory mediators in the development of anti-GBM disease. Thus impairing leukocyte recruitment and monocyte/macrophage glomerular infiltrate by blocking the chemokine CXCL16 (with a polyclonal anti-CXCL16 antiserum), depleting CD8(+) cells, or treatment with an antibody to perforin resulted in a significantly reduced severity of anti-GBM-induced glomerular injury. These results suggest that CD8(+) cells play a role in glomerular injury as effector cells, in part through a perforin/granzyme-mediated pathway.

Although anti-GBM disease is considered a prototypical antibody-mediated disease, several lines of evidence point to an important role for T cells in the initiation or pathogenesis of this disease. In addition to the association with HLA class II antigens, the involvement of T cells in the development of the autoimmune response to the Col4α3NC1 is supported by studies of T cell proliferation in response to monomeric components of the GBM and synthetic oligopeptides. A nephritogenic T cell epitope of Col4α3NC1 was demonstrated to induce glomerulonephritis in WKY rats. Interestingly, cross-reactive peptides from human infection-related microbes could be identified that also induced severe proteinuria and modest to severe glomerulonephritis in immunized rats. One peptide derived from Clostridium botulinum also induced pulmonary hemorrhage.

CD4+CD25+ Treg cells may play an important role in regulating the immune response in anti-GBM disease. The transfer of Treg cells attenuates the development of proteinuria and glomerular damage without preventing the deposition of immune complexes in mice that were previously immunized with rabbit IgG and before an injection of rabbit anti-GBM serum. In humans, Treg cells may explain in part the uncommon occurrence of disease relapses, and the eventual disappearance of anti-GBM antibodies in patients even without immunosuppressant medications as peripheral GBM-specific Treg cells emerge in the convalescent period, while undetected at the time of acute presentation.

The role of complement in the pathogenesis of anti-GBM disease is evidenced by the deposition of C3 along the GBM and supported by studies of animal models in mice and rabbits. These studies suggest that the terminal components of complement do not play a major part in the pathogenesis of the disease except in leukocyte-depleted animals. Studies using the heterologous anti-GBM model in mice rendered completely deficient of complement components C3 or C4 revealed a protective effect of C3 deficiency more than that of C4 deficiency. In an “attenuated” mouse model of anti-GBM using a subnephritogenic dose of
rabbit anti-mouse GBM antibody albuminuria was absent in Fcγ chain deficient and reduced in C3 deficient mice. C1q- and C4-deficient mice did develop proteinuria suggestive of involvement of the alternative pathway of complement. The role of Fcγ receptors is also evidenced by the occurrence of severe lung hemorrhage in mice deficient for the inhibitory Fcγ 2b receptor treated with bovine type IV collagen.

REFERENCES

64. Schönermark U, Csernok E, Trabandt A, Hansen H, Gross WL. Circulating cytokines and soluble CD23, CD26...
65. Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. Blood 1989;73(6):1504–1512
86. Kalhuri R, Danoff TM, Okada H, Neilson EG. Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell–mediated immunity in mice. J Clin Invest 1997;100(9):2263–2275


