Pathogenesis of Goodpasture Syndrome: A Molecular Perspective

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Goodpasture (GP) syndrome is a form of anti–glomerular basement membrane (GBM) disease, in which autoantibodies bind to α3(IV) collagen in GBM causing rapidly progressive glomerulonephritis and pulmonary hemorrhage. The conformational GP epitopes have been mapped to 2 regions within the noncollagenous (NC1) domain of the α3(IV) chain. Recently, we described the molecular organization of the autoantigen in the native α3α4α5(IV) collagen network of the GBM. The crystal structure of the NC1 domain has revealed how the GP epitopes are sequestered in the native GBM. Further insight into the pathogenesis of disease has been obtained from better animal models. These advances provide a foundation for the development of new specific therapies.

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Antibody-mediated glomerular injury is a major cause of glomerulonephritis leading to end-stage renal disease, often requiring dialysis or transplantation. The glomerular basement membrane (GBM), an essential component of the renal filtration barrier, is the target of pathogenic autoantibodies in anti-GBM disease and a notable cause of primary glomerulonephritis. The classic form of anti-GBM antibody nephritis, designated as Goodpasture (GP) syndrome, is an autoimmune disease clinically characterized by rapid progressive glomerulonephritis, lung hemorrhage, and the presence of circulating and organ-bound autoantibodies to the α3(IV) collagen chain. The relative involvement of kidneys and lungs varies among patients, and the reason for this variability is not known. In the absence of overt lung symptoms, the syndrome is commonly referred to as anti-GBM disease. The designation of anti-GBM disease may be preferable whenever antibodies to α3(IV) collagen are found because the autoantigen is the same regardless of clinical presentations.

Although rare, anti-GBM disease is fatal in nearly half of untreated patients, making early diagnosis and prompt therapeutic intervention extremely important. The standard treatment consists of immunosuppressive therapy and plasmapheresis to remove the pathogenic antibodies. Patients with low serum creatinine levels (<500 mg/dL) and few (<50%) glomerular crescents have a much better prognosis for recovery with treatment than they did 35 years ago. Immunoadsorption also can be used successfully in patients with advanced renal failure. Unlike many other autoimmune diseases that exhibit frequent relapses, recurrence of anti-GBM disease in either the native kidneys or renal transplant is not unheard of but is less common.

Anti-GBM Autoantibodies (Goodpasture Antibodies)

Based on the identification of immunoglobulin (Ig)G deposits at sites of injury in the glomerular and alveolar basement membranes, anti-GBM disease has been classified as an antibody-mediated autoimmune disease. The central pathogenic role of anti-GBM autoantibodies (also known as Goodpasture antibodies) has been shown by the ability of circulating or kidney-eluted anti-GBM antibodies to transfer the disease to monkeys or to human kidney allografts. Moreover, removal of circulating antibodies is therapeutic, and the severity of disease correlates with the titer of anti-GBM antibodies.

Fixation of anti-GBM antibodies to the GBM initiates a type II inflammatory response, characterized by the activation of complement and release of chemotactic peptides that recruit polymorphonuclear leukocytes and monocytes. Anti-GBM antibodies are almost exclusively of the IgG isotype, rarely IgA or IgM, and restricted to IgG1 and IgG4 subclasses. This subclass restriction may have pathologic significance because only IgG1 but not IgG4 can fix complement and bind macro-
phages through its Fc region. Interestingly, in 2 patients with recurrent anti-GBM antibodies, the reappearance of IgG1 was associated with recurrent disease, whereas IgG4 was not.19

Demonstration of kidney-bound and circulating anti-GBM autoantibodies is necessary for the diagnosis of anti-GBM disease and to exclude other pulmonary-renal syndromes such as some forms of vasculitis like Wegener’s granulomatosis. Analysis of kidney biopsy specimens from anti-GBM patients by direct immunofluorescence reveals a characteristic smooth linear deposition of IgG along the GBM, which may be accompanied by C3 deposition in a segmental or interrupted pattern. Serum-circulating anti-GBM antibodies can be detected by indirect immunofluorescence (they stain the GBM in a linear pattern) or, better, by sensitive and specific immunoassays using purified autoantigen. Enzyme-linked immunosorbent assay kits for detection of anti-GBM autoantibodies have become commercially available21 and a highly sensitive assay based on a biosensor system can detect anti-GBM antibodies in anti-GBM sera that appear negative by standard immunoassays.22

THE ANTI-GBM AUTOANTIGEN (THE GOODPASTURE ANTIGEN)

All anti-GBM sera contain autoantibodies that recognize common antigenic determinants of restricted specificity. Early studies on the nature of the autoantigen revealed that anti-GBM antibodies bound to 27- and 54-kd peptides solubilized by collagenase digestion of GBM.23-25 Subsequently, these were identified as monomers and dimers of the noncollagenous (NC1) domain of type IV collagen.26-27 Anti-GBM antibodies were shown to be directed against a novel α3(IV) collagen chain, termed the Goodpasture antigen.28,29 This identity was confirmed by binding of anti-GBM antibodies to recombinant α3(IV) NC1 domains expressed in vitro.30-33 Analysis of sera from a large cohort of patients with anti-GBM antibody nephritis has established further the α3(IV) NC1 domain as the common target of autoantibodies from Goodpasture patients with various clinical presentations.6

Lower titers of antibodies to additional GBM components, in particular to the NC1 domains of other collagen IV chains, are found in some anti-GBM sera,32,34,35 but whether they constitute epiphenomena or have pathogenic significance is unknown. In rare cases in which such antibodies have been identified in the absence of anti-α3(IV) antibodies, they are associated with other clinical phenotypes. For instance, antibodies to the α1(IV) NC1 domain are associated with paraneoplastic syndromes,36 and antibodies to the α5(IV) NC1 domain have been implicated in the pathogenesis of glomerulonephritis associated with skin blistering.37

The Epitopes of Anti-GBM Autoantibodies (the Goodpasture Epitopes)

Anti-GBM antibodies recognize conformational, disulfide-bond–dependent epitopes within the α3(IV) NC1 domain.25-36 This property made it difficult initially to map the epitopes using linear synthetic peptides.39,40 Subsequently, conformational epitopes were mapped using chimeric α1/α3 NC1 domains expressed in mammalian cells to ensure the correct folding. Two adjacent conformational anti-GBM epitopes designated E_A and E_B (Fig 1) have been proposed at residues 17-31 and 127-141 of the α3(IV) NC1 domain.33,41 Critical amino acids in the E_A epitope have been suggested,42,43 and several autoantibody subpopulations with distinct epitope specificities have been purified and characterized.44 Among these, GP_A antibodies, reacting with the E_A region, appear to be pathogenic because they are immunodominant in all anti-GBM patient sera and have the highest affinity for autoantigen. Moreover, an unfavorable disease outcome might correlate with high titers of autoantibodies toward the E_A but not the E_B region.45

The limited organ involvement in anti-GBM disease is related to the restricted specificity of the autoantibodies, the tissue-specific distribution of the α3(IV) collagen chain, and the accessibility of the antigen in glomerular and alveolar capillaries. The α3(IV) chain occurs in the basement membranes of the glomerulus, alveoli, eye, cochlea, choroid plexus, and testis. The prevalence of renal involvement in anti-GBM disease may be explained by the special structure of the glomerular capillaries, lined by a fenestrated endothelium that allows access of circulating antibodies to the GBM. With the exception of the lungs, other tissues containing the autoantigen are not obviously affected in anti-GBM disease, presumably because autoantibodies have limited access to the underly-
ing basement membranes, or the sites may be privileged by other regulatory mechanisms. Pulmonary hemorrhage in patients with anti-GBM disease is associated with smoking,46 or other factors that may permeabilize the integrity of lung capillaries, allowing anti-GBM autoantibodies to contact alveolar basement membrane.47

Quaternary Organization of the Anti-GBM Antigen

Type IV collagen occurs in basement membranes as supramolecular networks built by self-assembly of triple helical protomers (molecules) composed of 3 chains.48 An α3α4α5(IV) network (Fig 2) identified in the GBM represents the native form of the anti-GBM autoantigen.49,50 This network is essential for the structural integrity of the GBM and long-term maintenance of glomerular filtration.51 The α3α4α5(IV) network is composed of α3α4α5(IV) triple-helical protomers (Goodpasture protomers), which interact with 4 others through the amino termini forming a 7S domain, and dimerize with a partner through the carboxy-terminal NC1 domains forming an (α3α4α5)2 NC1 hexamer.2 This network is extensively cross-linked by intermolecular disulfide bonds along the protomers but not in the NC1 domains.52 The (α3α4α5)2 NC1 hexamer also is stabilized by interprotomer cross-links that convert a large proportion of NC1 monomers to α3-α5 and α4-α4 NC1 dimers.

A structural model of the (α3α4α5)2 NC1 hexamer has been proposed based on its homology to the crystallized ([α1]2[α2])2 NC1 hexamer harvested from lens capsule and placenta basement membranes.53,54 An NC1 hexamer is formed by 2 interfacing trimeric caps, each derived from 3 protomers, interacting through large planar surfaces. Within each trimeric cap, 3 NC1 domains are arranged radially around a 3-fold symmetric axis, so that the N-terminal half of each NC1 monomer interacts with C-terminal half of another monomer. Within the α3α4α5(IV) protomer, the E_A and E_B

![Figure 1](https://example.com/figure1.png)
epitopes of α3(IV) NC1 domain are located away from the protomer-protomer interface, and adjacent to the intraprotomer interfaces with the α5(IV) and α4(IV) NC1 domains, respectively. A surprising finding of the crystallographic studies was the absence of interdomain disulfide bonds, previously believed to cross-link the NC1 hexamers. Instead, a novel cross-link has been proposed involving the evolutionarily conserved Met residues and Lys residues. Cross-linking of α3(IV) NC1 monomers to α3-α5(IV) NC1 dimers contributes substantially to the sequestration of the anti-GBM autoepitopes within the native NC1 hexamer complex (see later).

Cryptic Properties of Goodpasture Epitopes

Unless dissociated, the Goodpasture epitopes are sequestered and inaccessible to anti-GBM autoantibodies binding in the native (α3α4α5)2 NC1 hexamers of the GBM. The epitopes are exposed reversibly in vitro under dissociating conditions, such as low pH level, sodium dodecyl sulfate, or...
logic conditions, causing hexamer dissociation. NC1 hexamers from human GBM under physiological conditions can react with native NC1 domains within the NC1 hexamer complex. Recent insight into the quaternary organization of the α3(IV) collagen chain confirms the accessibility of certain epitope residues in the Eα region decreases on formation of the NC1 hexamer. The epitope’s crypticity may provide important clues to the etiology and pathogenesis of anti-GBM disease. Restriction of the anti-GBM antibody response to sequenced, immunologically privileged epitopes suggests that a natural state of tolerance exists toward the native form of the autoantigen, but cryptic epitopes exposed by yet unidentified pathogenic processes will be perceived as new by the immune system. Presumably, an environmental factor, such as exposure to hydrocarbons or tobacco smoke is the stimulant needed to unmask the epitope in susceptible individuals. Reactive oxygen species expose cryptic anti-GBM epitopes in vitro, thus endogenous oxidants may play a role in opening these privileged sites in vivo. Oxidative stress can damage proteins directly or by increasing their susceptibility to proteases. Activated neutrophils also produce reactive oxygen species and secrete proteases that can damage the GBM. In this context, it is intriguing that about 25% of patients with anti-GBM disease also have antineutrophil cytoplasmic antibodies, primarily against myeloperoxidase. These patients probably have a vasculitis variant.

How anti-GBM autoantibodies bind cryptic epitopes in the GBM—a key pathogenic event—is not understood fully. In vitro, some species of anti-GBM autoantibodies can react with native NC1 hexamers from human GBM under physiologic conditions, causing hexamer dissociation. Intriguingly, only the α3(IV) NC1 monomers of the native NC1 hexamers react with anti-GBM antibodies under these conditions, even though the α3(IV) NC1 dimers are more abundant, suggesting that in vivo, pathogenic autoantibodies target only a susceptible subpopulation of α3(IV) chains.

ROLE OF CELLULAR IMMUNITY

Less is known about the role of cellular immunity in the pathogenesis of anti-GBM disease. CD4⁺ lymphocytes and macrophages can be found in the kidneys of patients with anti-GBM disease. Helper T cells clearly must play a role to drive the robust autoantibody response, as shown for other B-cell–dependent autoimmune diseases. Anti-GBM autoantibodies from various patients share private idiotypes, suggesting that the B-cell repertoire in this disease is highly regulated by CD4⁺ helper T cells. Differential susceptibility to anti-GBM disease in humans has been linked to class II major histocompatibility complex haplotypes mapping to the human leukocyte antigen DRB1 locus. Strong positive associations exist with human leukocyte antigen DRB1*1501 and DRB1*04 alleles, whereas DRB1*07 appears to be protective. This may reflect the differences in ability of class II molecules to bind autoantigen-derived peptides and present them to T helper cells. The human thymus expresses α3(IV) NC1 domain peptides that can negatively select for autoreactive CD4⁺ helper T cells. However, a few T cells may escape deletion during thymic editing and subsequently engage during induction of active disease. Major histocompatibility complex presentation of α3(IV) NC1 peptides may variably induce these T cells to help in the production of anti-GBM antibodies.

ALPORT POSTTRANSPLANT ANTI-GBM ANTIBODY NEPHRITIS

Mutations in the genes encoding the α3, α4, or α5(IV) collagen chains prevent the assembly of the α3α4α5(IV) collagen network causing Alport syndrome, a form of hereditary nephritis accompanied by sensorineural hearing loss and ocular abnormalities. The disease progresses slowly to end-stage renal disease, requiring dialysis or transplantation. Although all transplant recipients with Alport syndrome develop anti-GBM alloantibodies that bind to NC1 domains of the α3·α4·α5(IV) network, which is present in the allograft GBM but absent from the Alport GBM, only a small percentage of recipients develop anti-GBM nephritis that leads to loss of allograft function. Alport posttransplant anti-GBM nephritis is expressed variably, probably depending on cell-mediated susceptibility genes of the recipient.
In general, posttransplant alloantibodies from patients with COL4A5 mutations bind to the α5(IV) NC1 domain and those from patients with COL4A3 mutations bind to the α3(IV) NC1 domain.76-79 Despite many similarities between anti-GBM autoantibodies and Alport alloantibodies and the diseases they mediate, there is one significant difference. Although dissociation of GBM hexamers augments the binding of anti-GBM autoantibodies, it reduces the binding of Alport alloantibodies.44,47,58 These observations suggest that unlike the cryptic anti-GBM autoepitopes, the Alport allo-epitopes are accessible on the surface of the NC1 hexamers. This difference can be attributed to a prior absence of immune tolerance toward the native α3α4α5(IV) collagen network in patients with Alport syndrome. In contrast, in Goodpasture syndrome, immune tolerance toward the native form of the antigen must be evaded by exposure of cryptic epitopes, as discussed earlier.

**ANIMAL MODELS OF ANTI-GBM NEPHRITIS**

Anti-GBM antibody nephritis can be induced experimentally in numerous animal models either by passive transfer of anti-GBM antibodies or by immunization with nephritogenic antigen — usually prepared from type IV collagen from GBM. These observations stem from the seminal discovery by Steblay80 that immunization with human GBM induces autoimmune glomerulonephritis in sheep, a finding that set the stage for the subsequent recognition of the role of humoral autoimmunity in the pathogenesis of human anti-GBM disease. However, all studies of heterogeneous mixtures of nephritogenic antigens (sometimes across species) cannot be extrapolated directly to human anti-GBM disease, in which the autoantibody specificity is rather restricted.

After identification of α3(IV) NC1 domain as the human anti-GBM autoantigen, animal models of anti-GBM nephritis were refined by using purified preparations of antigen. The animal model of choice, the Wistar-Kyoto (WKY) strain of rats, is susceptible to develop experimental anti-GBM nephritis recapitulating the clinical features of human anti-GBM disease after a single immunization with nephritogenic antigen. On challenge with NC1 domains isolated from autologous or heterologous GBM by collagenase digestion, WKY rats produce anti-GBM antibodies that transfer nephritis passively.81 develop glomerulonephritis characterized by proteinuria, hematuria, and linear deposition of IgG along GBM, and exhibit pulmonary hemorrhage.82-85 Only purified fractions containing the α3-α5(IV) NC1 domains from human, bovine, and rat GBM, but not the α1 and α2(IV) NC1 domains were nephritogenic in rats.86-90 Purified α3(IV) NC1 dimers also induce anti-GBM disease in rabbits91 and mice.92 Induction of autoimmune glomerulonephritis in WKY rats by recombinant human or rat α3(IV) NC1 monomers93-95 unambiguously established the pathogenic role of anti-GBM autoantigen in both human anti-GBM disease and its animal models. Intriguingly, the α4(IV) NC1 domains also induced severe nephritis in rats, whereas other NC1 domains were not pathogenic, except for the α5(IV) NC1 domain, which produced mild disease.93 Attempts to identify the nephritogenic anti-GBM epitopes by immunizing rats with α3 NC1-derived peptides could not induce anti-GBM nephritis reliably,96-98 suggesting that disease induction may require multiple epitopes, native folding, and/or posttranslational processing of the autoantigen.

Recent evidence from rodent models of anti-GBM disease suggests an important role for cellular immunity. Anti-GBM disease could be induced by adoptive transfer of CD4+ T cells from rats immunized with α3(IV) NC1 domain.99 In a mouse model, crescentic glomerulonephritis and lung hemorrhage were associated with the emergence of an interleukin 12/Th1-like T-cell phenotype that was major histocompatibility complex class II restricted.92 Murine H-2a, d, and b haplotypes, but not H-2k nor a, developed the glomerulonephritis and pulmonary hemorrhage after immunization with α3(IV) NC1 dimers, although all haplotypes developed anti-α3(IV) NC1 antibodies. Lymphocytes or anti-GBM antibodies from nephritogenic strains transfer disease to syngeneic recipients, but passive transfer of anti-α3(IV) NC1 antibodies into T-cell receptor-deficient mice failed to produce nephritis.92 In both mouse and rat models, tolerance to experimental anti-GBM disease can be induced by oral administration of autoantigen92,100 although not by intrathymic injection of α3(IV) NC1 domain.90 Finally, anti-GBM disease can be attenuated or prevented by monoclonal antibodies to adhesion molecules intercellular adhesion molecule 1 and leukocyte function antigen-1 (LFA-
1),\textsuperscript{101} by anti-CD4 antibodies to helper T cells,\textsuperscript{102} or by the blockade of the CD28-B7 costimulatory pathway.\textsuperscript{103} It is expected that animal models of anti-GBM nephritis will continue to provide insight into the molecular mechanism underlying the etiology and pathogenesis of human anti-GBM disease, and will be instrumental for developing and testing new therapeutic approaches.

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