Pathogenesis of Goodpasture Syndrome: A Molecular Perspective

By Dorin-Bogdan Borza, Eric G. Neilson, and Billy G. Hudson

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.

ANTIBODY-MEDIATED GLOMERULAR injury is a major cause of glomerulonephritis leading to end-stage renal disease, often requiring dialysis or transplantation.[1] The glomerular basement membrane (GBM), an essential component of the renal filtration barrier,[2] is the target of pathogenic autoantibodies in anti-GBM disease and a notable cause of primary glomerulonephritis.[3] The classic form of anti-GBM antibody nephritis, designated as *Goodpasture (GP) syndrome*,[4] 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,[5] 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.[6]

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.[7] 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.[8,9] Immunoadsorption also can be used successfully in patients with advanced renal failure.[10] 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.[11-13]

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,[14-16] 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.[5,12,17] Moreover, removal of circulating antibodies is therapeutic, and the severity of disease correlates with the titer of anti-GBM antibodies.[18]

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.[3] Anti-GBM antibodies are almost exclusively of the IgG isotype, rarely IgA or IgM, and restricted to IgG1 and IgG4 subclasses.[19,20] This subclass restriction may have pathologic significance because only IgG1 but not IgG4 can fix complement and bind macro-

From the Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.

Supported in part by National Institutes of Health grants DK65123 (D.-B.B.), DK18381 and DK53763 (B.G.H.), DK46282 and DK55926 (E.G.N.), and the 2003 Carl Gottschalk Research Scholar Award from the American Society of Nephrology (D.-B.B.).

Address reprint requests to Dorin-Bogdan Borza, Division of Nephrology, S-3223 Medical Center North, Vanderbilt University Medical Center; 1161 21st Ave South, Nashville, TN 37232-2372. Email: Dorin-Bogdan.Borza@vanderbilt.edu

0270-9295/03/2306-0003$30.00/0
doi:10.1053/S0270-9295(03)00131-1

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 available[21] 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.[25,38] 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-

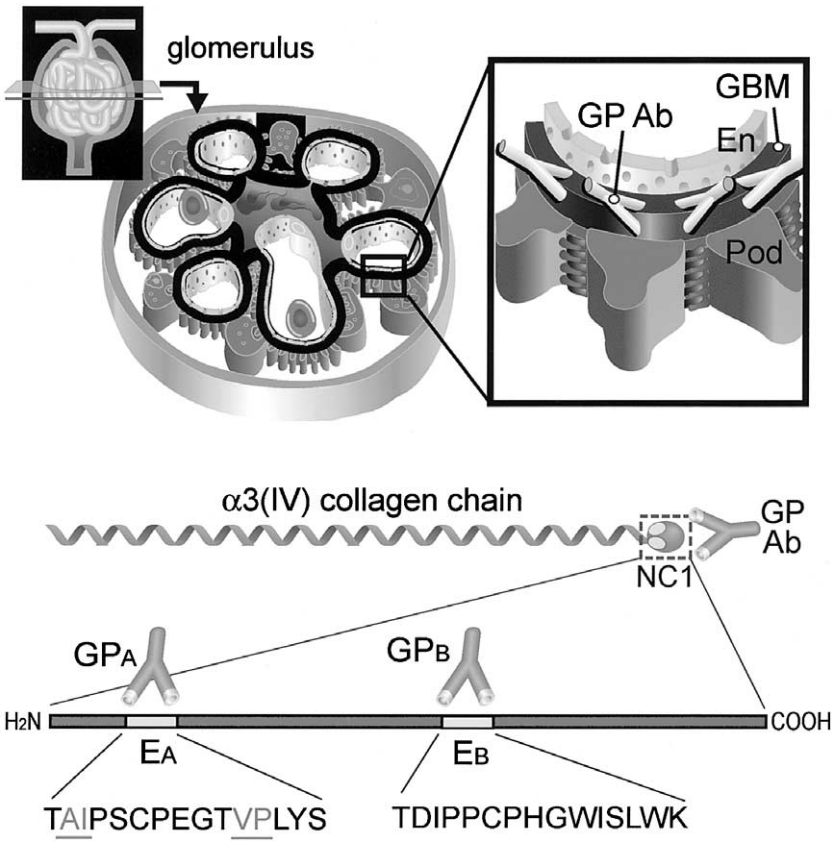

Fig 1. The GBM is located between the glomerular visceral epithelial cells (podocytes [POD]) and endothelial cells (EN), and is the target of pathogenic anti-GBM antibodies (AB) in Goodpasture syndrome. Anti-GBM autoantibodies bind to 2 regions within the NC1 domain of the α3(IV) collagen chain, designated E_A and E_B. Within the E_A region, several hydrophobic amino acids were found critical for binding of GP_A antibodies (underlined).

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 $\alpha3\alpha4\alpha5$(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 $\alpha3\alpha4\alpha5$(IV) network is composed of $\alpha3\alpha4\alpha5$(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 carboxyl-terminal NC1 domains forming an $(\alpha3\alpha4\alpha5)_2$ NC1 hexamer.[2] This network is extensively cross-linked by intermolecular disulfide bonds along the protomers but not in the NC1 domains.[52] The $(\alpha3\alpha4\alpha5)_2$ NC1 hexamer also is stabilized by interprotomer cross-links that convert a large proportion of NC1 monomers to $\alpha3$-$\alpha5$ and $\alpha4$-$\alpha4$ NC1 dimers.

A structural model of the $(\alpha3\alpha4\alpha5)_2$ NC1 hexamer has been proposed based on its homology to the crystallized $([\alpha1]_2[\alpha2])_2$ 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 $\alpha3\alpha4\alpha5$(IV) protomer, the E_A and E_B

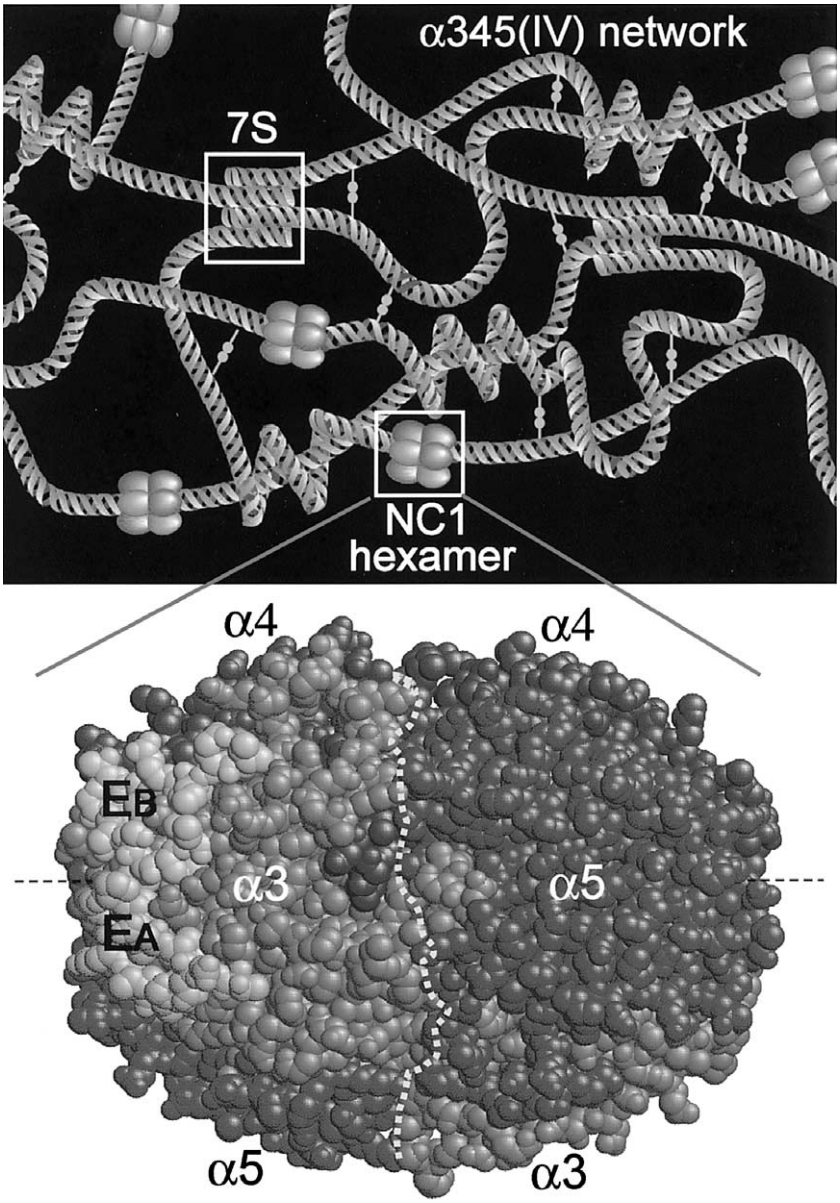

Fig 2. Anti-GBM autoantigen is found in the native GBM as an $\alpha3\alpha4\alpha5$(IV) network composed of $\alpha3\alpha4\alpha5$ (IV) protomers (top). The protomers interact through their amino termini forming a 7S domain, dimerize through their carboxyl termini forming an $(\alpha3\alpha4\alpha5)_2$ NC1 hexamer (bottom), and are cross-linked extensively by intermolecular disulfide bonds. Within the NC1 hexamer, the E_A and E_B regions of the $\alpha3$(IV) NC1 domain, which encompass the epitopes of anti-GBM antibodies, are distant from the protomer-protomer interface (dotted line), but adjacent to the $\alpha5$(IV) and $\alpha4$(IV) NC1 domains, respectively. Dissociation of the NC1 hexamer is required to expose the cryptic anti-GBM epitopes and allow binding of autoantibodies.

epitopes of $\alpha3$(IV) NC1 domain are located away from the protomer-protomer interface, and adjacent to the intraprotomer interfaces with the $\alpha5$(IV) and $\alpha4$(IV) NC1 domains, respectively.[52] A surprising finding of the crystallographic studies was the absence of interdomain disulfide bonds, previously believed to cross-link the NC1 hexamers.[55] Instead, a novel cross-link has been proposed involving the evolutionarily conserved Met^{93} and Lys^{211} residues.[54] Cross-linking of $\alpha3$(IV) NC1 monomers to $\alpha3$-$\alpha5$(IV) NC1 dimers contributes substantially to the sequestration of the anti-GBM autoepitopes within the native NC1 hexamer complex (see later).[44]

Cryptic Properties of Goodpasture Epitopes

Unless dissociated, the Goodpasture epitopes are sequestered and inaccessible to anti-GBM autoantibodies binding in the native $(\alpha3\alpha4\alpha5)_2$ NC1 hexamers of the GBM.[44] The epitopes are exposed reversibly in vitro under dissociating conditions, such as low pH level, sodium dodecyl sulfate, or

guanidine.[56] In immunofluorescence,[57] enzyme-linked immunosorbent assay,[58] or immunoprecipitation assays,[44] binding of anti-GBM autoantibodies to dissociated hexamers is increased several-fold, compared with native hexamers. The finding that several hydrophobic amino acids are critical for binding GP_A antibodies provided a molecular basis for the cryptic properties; the E_A epitope may be sequestered by hydrophobic interactions among NC1 domains within the NC1 hexamer complex.[42] Recent insight into the quaternary organization of the α3(IV) collagen chain confirms the accessibility of certain epitope residues in the E_A region decreases on formation of the NC1 hexamer.[52]

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 sequestered, 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[59,60] or tobacco smoke[46] is the stimulant needed to unmask the epitope in susceptible individuals. Reactive oxygen species expose cryptic anti-GBM epitopes in vitro,[47] 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.[61] Activated neutrophils also produce reactive oxygen species and secrete proteases that can damage the GBM.[62-64] In this context, it is intriguing that about 25% of patients with anti-GBM disease also have antineutrophil cytoplasmic antibodies, primarily against myeloperoxidase.[65,66] 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.[44] 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.[67] 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.[68] 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.[69-71] 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.[72] 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.[73]

ALPORT POSTTRANSPLANT ANTI-GBM ANTIBODY NEPHRITIS

Mutations in the genes encoding the α3, α4, or α5(IV) collagen chains prevent the assembly of the $\alpha3\alpha4\alpha5$(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 $\alpha3 \cdot \alpha4 \cdot \alpha5$(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.[74] Alport posttransplant anti-GBM nephritis is expressed variably, probably depending on cell-mediated susceptibility genes of the recipient.[75]

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 Steblay[80] 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 rabbits[91] and mice.[92] Induction of autoimmune glomerulonephritis in WKY rats by recombinant human or rat α3(IV) NC1 monomers[93-95] 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-2^s, ^{d}, and ^{b} haplotypes, but not H-2^k 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 autoantigen,[92,100] 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),[101] by anti-CD4 antibodies to helper T cells,[102] or by the blockade of the CD28-B7 costimulatory pathway.[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.

REFERENCES

1. Wilson CB: Renal response to immunologic glomerular injury, in Brenner BM, (ed): The Kidney. 5th ed. Philadelphia, Saunders, 1996, vol 2:pp 1253-1391

2. Hamano Y, Grunkemeyer JA, Sudhakar A, et al: Determinants of vascular permeability in the kidney glomerulus. J Biol Chem 277:31154-31162, 2002

3. Hudson BG, Tryggvason K, Sundaramoorthy M, et al: Mechanisms of disease: Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med 348:2543-2556, 2003

4. Stanton MC, Tange JD: Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). Aust Ann Med 7:132-144, 1958

5. Wilson CB, Dixon FJ: Anti-glomerular basement membrane antibody-induced glomerulonephritis. Kidney Int 3:74-89, 1973

6. Kalluri R, Wilson CB, Weber M, et al: Identification of the alpha 3 chain of type IV collagen as the common autoantigen in antibasement membrane disease and Goodpasture syndrome. J Am Soc Nephrol 6:1178-1185, 1995

7. Lockwood CM, Rees AJ, Pearson TA, et al: Immunosuppression and plasma-exchange in the treatment of Goodpasture's syndrome. Lancet 1:711-715, 1976

8. Merkel F, Pullig O, Marx M, et al: Course and prognosis of anti-basement membrane antibody (anti-BM-Ab)- mediated disease: Report of 35 cases. Nephrol Dial Transplant 9:372-376, 1994

9. Levy JB, Turner AN, Rees AJ, et al: Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. Ann Intern Med 134:1033-1042, 2001

10. Laczika K, Knapp S, Derfler K, et al: Immunoadsorption in Goodpasture's syndrome. Am J Kidney Dis 36:392-395, 2000

11. Dahlberg PJ, Kurtz SB, Donadio JV, et al: Recurrent Goodpasture's syndrome. Mayo Clin Proc 53:533-537, 1978

12. Almkuist RD, Buckalew VM Jr, Hirszel P, et al: Recurrence of anti-glomerular basement membrane antibody mediated glomerulonephritis in an isograft. Clin Immunol Immunopathol 18:54-60, 1981

13. Levy JB, Lachmann RH, Pusey CD: Recurrent Goodpasture's disease. Am J Kidney Dis 27:573-578, 1996

14. Scheer RL, Grossman MA: Immune aspects of glomerulonephritis associated with pulmonary hemorrhage. Ann Intern Med 60:1009, 1964

15. Duncan DA, Drummond KN, Richard AF, et al: Pulmonary hemorrhage and glomerulonephritis: Report of six cases and study of the renal lesions by the fluorescent antibody technique and electron microscopy. Ann Intern Med 62:920-938, 1965

16. Sturgill BC, Westervelt FB: Immunofluorescence studies in a case of Goodpasture's syndrome. JAMA 194:914-916, 1965

17. Lerner RA, Glassock RJ, Dixon FJ: The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. J Exp Med 126:989-1004, 1967

18. Herody M, Bobrie G, Gouarin C, et al: Anti-GBM disease: Predictive value of clinical, histological and serological data. Clin Nephrol 40:249-255, 1993

19. Bowman C, Ambrus K, Lockwood CM: Restriction of human IgG subclass expression in the population of autoantibodies to glomerular basement membrane. Clin Exp Immunol 69:341-349, 1987

20. Noel LH, Aucouturier P, Monteiro RC, et al: Glomerular and serum immunoglobulin G subclasses in membranous nephropathy and anti-glomerular basement membrane nephritis. Clin Immunol Immunopathol 46:186-194, 1988

21. Jaskowski TD, Martins TB, Litwin CM, et al: Comparison of four enzyme immunoassays for the detection of immunoglobulin G antibody against glomerular basement membrane. J Clin Lab Anal 16:143-145, 2002

22. Salama AD, Dougan T, Levy JB, et al: Goodpasture's disease in the absence of circulating anti-glomerular basement membrane antibodies as detected by standard techniques. Am J Kidney Dis 39:1162-1167, 2002

23. Wilson CB, Holdsworth SR, Neale TJ: Anti-basement membrane antibodies in immunologic renal disease. Aust N Z J Med 11:94-100, 1981

24. Fish AJ, Lockwood MC, Wong MDetection of Goodpasture antigen in fractions prepared from collagenase digests of human glomerular basement membrane: Clin Exp Immunol 55:58-66, 1984

25. Wieslander J, Bygren P, Heinegard D: Isolation of the specific glomerular basement membrane antigen involved in Goodpasture syndrome. Proc Natl Acad Sci U S A 81:1544-1548, 1984

26. Wieslander J, Barr JF, Butkowski RJ, et al: Goodpasture antigen of the glomerular basement membrane: Localization to noncollagenous regions of type IV collagen. Proc Natl Acad Sci U S A 81:3838-3842, 1984

27. Butkowski RJ, Wieslander J, Wisdom BJ, et al: Properties of the globular domain of type IV collagen and its relationship to the Goodpasture antigen. J Biol Chem 260:3739-3747, 1985

28. Butkowski RJ, Langeveld JP, Wieslander J, et al: Localization of the Goodpasture epitope to a novel chain of basement membrane collagen. J Biol Chem 262:7874-7877, 1987

29. Saus J, Wieslander J, Langeveld JP, et al: Identification of the Goodpasture antigen as the alpha 3(IV) chain of collagen IV. J Biol Chem 263:13374-13380, 1988

30. Neilson EG, Kalluri R, Sun MJ, et al: Specificity of Goodpasture autoantibodies for the recombinant noncollagenous domains of human type IV collagen. J Biol Chem 268: 8402-8405, 1993

31. Turner N, Forstova J, Rees A, et al: Production and characterization of recombinant Goodpasture antigen in insect cells. J Biol Chem 269:17141-17145, 1994

32. Dehan P, Weber M, Zhang X, et al: Sera from patients with anti-GBM nephritis including Goodpasture syndrome show heterogenous reactivity to recombinant NC1 domain of type IV collagen alpha chains. Nephrol Dial Transplant 11: 2215-2222, 1996

33. Netzer KO, Leinonen A, Boutaud A, et al: The Goodpasture autoantigen. Mapping the major conformational epitope(s) of alpha3(IV) collagen to residues 17-31 and 127-141 of the NC1 domain. J Biol Chem 274:11267-11274, 1999

34. Segelmark M, Butkowski R, Wieslander J: Antigen restriction and IgG subclasses among anti-GBM autoantibodies. Nephrol Dial Transplant 5:991-996, 1990

35. Hellmark T, Johansson C, Wieslander J: Characterization of anti-GBM antibodies involved in Goodpasture's syndrome. Kidney Int 46:823-829, 1994

36. Kalluri R, Petrides S, Wilson CB, et al: Anti-alpha1(IV) collagen autoantibodies associated with lung adenocarcinoma presenting as the Goodpasture syndrome. Ann Intern Med 124: 651-653, 1996

37. Ghohestani RF, Hudson BG, Claudy A, et al: The alpha 5 chain of type IV collagen is the target of IgG autoantibodies in a novel autoimmune disease with subepidermal blisters and renal insufficiency. J Biol Chem 275:16002-16006, 2000

38. Kalluri R, Gunwar S, Reeders ST, et al: Goodpasture syndrome. Localization of the epitope for the autoantibodies to the carboxyl-terminal region of the alpha 3(IV) chain of basement membrane collagen. J Biol Chem 266:24018-24024, 1991

39. Hellmark T, Brunmark C, Trojnar J, et al: Epitope mapping of anti-glomerular basement membrane (GBM) antibodies with synthetic peptides. Clin Exp Immunol 105:504-510, 1996

40. Levy JB, Coulthart A, Pusey CD: Mapping B cell epitopes in Goodpasture's disease. J Am Soc Nephrol 8:1698-1705, 1997

41. Hellmark T, Burkhardt H, Wieslander J: Goodpasture disease. Characterization of a single conformational epitope as the target of pathogenic autoantibodies. J Biol Chem 274: 25862-25868, 1999

42. David M, Borza DB, Leinonen A, et al: Hydrophobic amino acid residues are critical for the immunodominant epitope of the Goodpasture autoantigen. A molecular basis for the cryptic nature of the epitope. J Biol Chem 276:6370-6377, 2001

43. Gunnarsson A, Hellmark T, Wieslander J: Molecular properties of the Goodpasture epitope. J Biol Chem 275:30844-30848, 2000

44. Borza DB, Netzer KO, Leinonen A, et al: The Goodpasture autoantigen: Identification of multiple cryptic epitopes on the NC1 domain of the alpha3(IV) collagen chain. J Biol Chem 275:6030-6037, 2000

45. Hellmark T, Segelmark M, Unger C, et al: Identification of a clinically relevant immunodominant region of collagen IV in Goodpasture disease. Kidney Int 55:936-944, 1999

46. Donaghy M, Rees AJ: Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. Lancet 2:1390-1393, 1983

47. Kalluri R, Cantley LG, Kerjaschki D, et al: Reactive oxygen species expose cryptic epitopes associated with autoimmune Goodpasture syndrome. J Biol Chem 275:20027-20032, 2000

48. Timpl R, Wiedemann H, van Delden V, et al: A network model for the organization of type IV collagen molecules in basement membranes. Eur J Biochem 120:203-211, 1981

49. Gunwar S, Ballester F, Noelken ME, et al: Glomerular basement membrane. Identification of a novel disulfide-cross-linked network of a3, a4 and a5 chains of type IV collagen and its implications for the pathogenesis of Alport syndrome. J Biol Chem 273:8767-8775, 1998

50. Boutaud A, Borza DB, Bondar O, et al: Type IV collagen of the glomerular basement membrane: Evidence that the chain specificity of network assembly is encoded by the noncollagenous NC1 domains. J Biol Chem 275:30716-30724, 2000

51. Kalluri R, Shield CF, Todd P, et al: Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. J Clin Invest 99:2470-2478, 1997

52. Borza DB, Bondar O, Todd P, et al: Quaternary organization of the Goodpasture autoantigen, the a3(IV) collagen chain: Sequestration of two cryptic autoepitopes by intra-protomer interactions with the a4 and a5 NC1 domains. J Biol Chem 277:40075-40083, 2002

53. Sundaramoorthy M, Meiyappan M, Todd P, et al: Crystal structure of NC1 domains: Structural basis for type IV collagen assembly in basement membranes. J Biol Chem 277:31142-31153, 2002

54. Than ME, Henrich S, Huber R, et al: The 1.9-A crystal structure of the noncollagenous (NC1) domain of human placenta collagen IV shows stabilization via a novel type of covalent Met-Lys cross-link. Proc Natl Acad Sci U S A 99:6607-6612, 2002

55. Siebold B, Deutzmann R, Kuhn K: The arrangement of intra- and intermolecular disulfide bonds in the carboxyterminal, non-collagenous aggregation and cross-linking domain of basement-membrane type IV collagen. Eur J Biochem 176:617-624, 1988

56. Wieslander J, Langeveld J, Butkowski R, et al: Physical and immunochemical studies of the globular domain of type IV collagen. Cryptic properties of the Goodpasture antigen. J Biol Chem 260:8564-8570, 1985

57. Yoshioka K, Michael AF, Velosa J, et al: Detection of hidden nephritogenic antigen determinants in human renal and nonrenal basement membranes. Am J Pathol 121:156-165, 1985

58. Kalluri R, Sun MJ, Hudson BG, et al: The Goodpasture autoantigen. Structural delineation of two immunologically privileged epitopes on alpha3(IV) chain of type IV collagen. J Biol Chem 271:9062-9068, 1996

59. Stevenson A, Yaqoob M, Mason H, et al: Biochemical markers of basement membrane disturbances and occupational exposure to hydrocarbons and mixed solvents. QJM 88:23-28, 1995

60. Bombassei GJ, Kaplan AA: The association between hydrocarbon exposure and anti-glomerular basement membrane antibody-mediated disease (Goodpasture's syndrome). Am J Ind Med 21:141-153, 1992

61. Stadtman ER: Role of oxidized amino acids in protein breakdown and stability. Methods Enzymol 258:379-393, 1995

62. Vissers MC, Winterbourn CC, Hunt JS: Degradation of

glomerular basement membrane by human neutrophils in vitro. Biochim Biophys Acta 804:154-160, 1984

63. Vissers MC, Day WA, Winterbourn CC: Neutrophils adherent to a nonphagocytosable surface (glomerular basement membrane) produce oxidants only at the site of attachment. Blood 66:161-166, 1985

64. Donovan KL, Davies M, Coles GA, et al: Relative roles of elastase and reactive oxygen species in the degradation of human glomerular basement membrane by intact human neutrophils. Kidney Int 45:1555-1561, 1994

65. Weber MF, Andrassy K, Pullig O, et al: Antineutrophil-cytoplasmic antibodies and antiglomerular basement membrane antibodies in Goodpasture's syndrome and in Wegener's granulomatosis. J Am Soc Nephrol 2:1227-1234, 1992

66. Hellmark T, Niles JL, Collins AB, et al: Comparison of anti-GBM antibodies in sera with or without ANCA. J Am Soc Nephrol 8:376-385, 1997

67. Derry CJ, Ross CN, Lombardi G, et al: Analysis of T cell responses to the autoantigen in Goodpasture's disease. Clin Exp Immunol 100:262-268, 1995

68. Meyers KE, Kinniry PA, Kalluri R, et al: Human Goodpasture anti-alpha3(IV)NC1 autoantibodies share structural determinants. Kidney Int 53:402-407, 1998

69. Fisher M, Pusey CD, Vaughan RW, et al: Susceptibility to anti-glomerular basement membrane disease is strongly associated with HLA-DRB1 genes. Kidney Int 51:222-229, 1997

70. Huey B, McCormick K, Capper J, et al: Associations of HLA-DR and HLA-DQ types with anti-GBM nephritis by sequence-specific oligonucleotide probe hybridization. Kidney Int 44:307-312, 1993

71. Phelps RG, Rees AJ: The HLA complex in Goodpasture's disease: A model for analyzing susceptibility to autoimmunity. Kidney Int 56:1638-1653, 1999

72. Wong D, Phelps RG, Turner AN: The Goodpasture antigen is expressed in the human thymus. Kidney Int 60:1777-1783, 2001

73. Salama AD, Chaudhry AN, Ryan JJ, et al: In Goodpasture's disease, CD4(+) T cells escape thymic deletion and are reactive with the autoantigen alpha3(IV)NC1. J Am Soc Nephrol 12:1908-1915, 2001

74. Milliner DS, Pierides AM, Holley KE: Renal transplantation in Alport's syndrome: Anti-glomerular basement membrane glomerulonephritis in the allograft. Mayo Clin Proc 57: 35-43, 1982

75. Kalluri R, Torre A, Shield CF 3rd, et al: Identification of alpha3, alpha4, and alpha5 chains of type IV collagen as alloantigens for Alport posttransplant anti-glomerular basement membrane antibodies. Transplantation 69:679-683, 2000

76. Hudson BG, Kalluri R, Gunwar S, et al: The pathogenesis of Alport syndrome involves type IV collagen molecules containing the alpha 3(IV) chain: Evidence from anti-GBM nephritis after renal transplantation. Kidney Int 42:179-187, 1992

77. Kalluri R, Weber M, Netzer KO, et al: COL4A5 gene deletion and production of post-transplant anti-alpha 3(IV) collagen alloantibodies in Alport syndrome. Kidney Int 45:721-726, 1994

78. Dehan P, Van den Heuvel LP, Smeets HJ, et al: Identification of post-transplant anti-alpha 5 (IV) collagen alloantibodies in X-linked Alport syndrome. Nephrol Dial Transplant 11:1983-1988, 1996

79. Brainwood D, Kashtan C, Gubler MC, et al: Targets of alloantibodies in Alport anti-glomerular basement membrane disease after renal transplantation. Kidney Int 53:762-766, 1998

80. Steblay RW: Glomerulonephritis induced in sheep by injections of heterologous glomerular basement membrane and Freund's complete adjuvant. J Exp Med 116:253-271, 1962

81. Sado Y, Naito I, Okigaki T: Transfer of anti-glomerular basement membrane antibody-induced glomerulonephritis in inbred rats with isologous antibodies from the urine of nephritic rats. J Pathol 158:325-332, 1989

82. Sado Y, Okigaki T, Takamiya H, et al: Experimental autoimmune glomerulonephritis with pulmonary hemorrhage in rats. The dose-effect relationship of the nephritogenic antigen from bovine glomerular basement membrane. J Clin Lab Immunol 15:199-204, 1984

83. Sado Y, Naito I, Akita M, et al: Strain specific responses of inbred rats on the severity of experimental autoimmune glomerulonephritis. J Clin Lab Immunol 19:193-199, 1986

84. Sado Y, Naito I: Experimental autoimmune glomerulonephritis in rats by soluble isologous or homologous antigens from glomerular and tubular basement membranes. Br J Exp Pathol 68:695-704, 1987

85. Bolton WK, May WJ, Sturgill BC: Proliferative autoimmune glomerulonephritis in rats: A model for autoimmune glomerulonephritis in humans. Kidney Int 44:294-306, 1993

86. Sado Y, Kagawa M, Naito I, et al: Properties of bovine nephritogenic antigen that induces anti-GBM nephritis in rats and its similarity to the Goodpasture antigen. Virchows Arch 60:345-351, 1991

87. Bolton WK, Luo AM, Fox PL, et al: Study of EHS type IV collagen lacking Goodpasture's epitope in glomerulonephritis in rats. Kidney Int 47:404-410, 1995

88. Rauf S, Kagawa M, Kishiro Y, et al: Nephritogenicity and alpha-chain composition of NC1 fractions of type IV collagen from bovine renal basement membrane. Virchows Arch 428:281-288, 1996

89. Sado Y, Kagawa M, Kishiro Y, et al: Purification and characterization of human nephritogenic antigen that induces anti-GBM nephritis in rats. J Pathol 182:225-232, 1997

90. Abbate M, Kalluri R, Corna D, et al: Experimental Goodpasture's syndrome in Wistar-Kyoto rats immunized with a3 chain of type IV collagen. Kidney Int 54:1550-1561, 1998

91. Kalluri R, Gattone VH 2nd, Noelken ME, et al: The alpha 3 chain of type IV collagen induces autoimmune Goodpasture syndrome. Proc Natl Acad Sci U S A 91:6201-6205, 1994

92. Kalluri R, Danoff TM, Okada H, et al: 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 100:2263-2275, 1997

93. Sado Y, Boutaud A, Kagawa M, et al: Induction of anti-GBM nephritis in rats by recombinant a3(IV)NC1 and a4(IV)NC1 of type IV collagen. Kidney Int 53:664-671, 1998

94. Ryan JJ, Reynolds J, Norgan VA, et al: Expression and characterization of recombinant rat alpha 3(IV)NC1 and its use

in induction of experimental autoimmune glomerulonephritis. Nephrol Dial Transplant 16:253-261, 2001

95. Wu J, Hicks J, Ou C, et al: Glomerulonephritis induced by recombinant collagen IV alpha 3 chain noncollagen domain 1 is not associated with glomerular basement membrane antibody: A potential T cell-mediated mechanism. J Immunol 167: 2388-2395, 2001

96. Luo AM, Fox JW, Chen L, et al: Synthetic peptides of Goodpasture's antigen in antiglomerular basement membrane nephritis in rats. J Lab Clin Med 139:303-310, 2002

97. Bolton WK, Luo AM, Fox P, et al: Goodpasture's epitope in development of experimental autoimmune glomerulonephritis in rats. Kidney Int 49:327-334, 1996

98. Sugihara K, Sado Y, Ninomiya Y, et al: Experimental anti-GBM glomerulonephritis induced in rats by immunization with synthetic peptides based on six alpha chains of human type IV collagen. J Pathol 178:352-358, 1996

99. Wu J, Hicks J, Borillo J, et al: CD4(+) T cells specific to a glomerular basement membrane antigen mediate glomerulonephritis. J Clin Invest 109:517-524, 2002

100. Reynolds J, Pusey CD: Oral administration of glomerular basement membrane prevents the development of experimental autoimmune glomerulonephritis in the WKY rat. J Am Soc Nephrol 12:61-70, 2001

101. Nishikawa K, Guo YJ, Miyasaka M, et al: Antibodies to intercellular adhesion molecule 1/lymphocyte function-associated antigen 1 prevent crescent formation in rat autoimmune glomerulonephritis. J Exp Med 177:667-677, 1993

102. Reynolds J, Pusey CD: In vivo treatment with a monoclonal antibody to T helper cells in experimental autoimmune glomerulonephritis in the BN rat. Clin Exp Immunol 95:122-127, 1994

103. Reynolds J, Tam FW, Chandraker A, et al: CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis. J Clin Invest 105:643-651, 2000