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Goodpasture's Disease: molecular architecture of the autoantigen provides clues to etiology and pathogenesis

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Abstract

Purpose of review—Goodpasture's (GP) disease is an autoimmune disorder characterized by the deposition of pathogenic autoantibodies in basement membranes of kidney and lung, which induces rapidly progressive glomerulonephritis and pulmonary hemorrhage. The target antigen is the α3NC1 domain of collagen IV, which is expressed in target organs as aα345 network. Recent studies of specificity and epitopes of GP autoantibodies and discovery of novel posttranslational modification of the antigen, a sulfilimine bond, provide further insight into mechanisms of initiation and progression of GP disease.

Recent findings—Analysis of the specificity of GP autoantibodies revealed distinct subset of circulating and kidney-bound anti-α5NC1 antibody, which is associated with loss of kidney function. Structural integrity of the α345NC1 hexamer is stabilized by the novel sulfilimine crosslinks conferring immune privilege to the GP autoantigen. Native antibodies may contribute to establishment of immune tolerance to autoantigen. Structural analysis of epitopes for autoantibodies and alloantibodies indicates a critical role of conformational change in the α345NC1 hexamer in eliciting of autoimmune response in GP disease.

Summary—Understanding of the quaternary structure of the GP autoantigen continues to provide insights into autoimmune mechanisms that serve as a basis for developing of novel diagnostic tools and therapies for Goodpasture's disease.

Keywords

Collagen type IV; glomerular basement membrane; Goodpasture's disease; autoantibodies; autoantigen; epitopes

Introduction

Over four decades of studies, Goodpasture's (GP) disease has emerged as a model for exploring mechanisms that underlie autoimmunity. GP disease is a rare disorder (1 case per 1 million per year [1]), which accounts for about 5% of all cases of glomerulonephritis in adults. However, if not treated promptly by combination of immunosuppression and plasma exchange, it results in acute renal failure with a fatal outcome in about a half of the patients [2,3]. Goodpasture's disease is an organ-specific autoimmune disorder characterized by

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rapidly progressive glomerulonephritis and linear deposits of antibodies along the glomerular basement membrane (GBM), sometimes with pulmonary hemorrhage induced by antibody binding to lung basement membranes. In the absence of pulmonary manifestations, the disease is often referred to as anti-GBM nephritis. For this review we use the term GP disease with an emphasis on circulating and tissue-bound anti-GBM autoantibodies, independent of clinical manifestations.

In a landmark study, Richard Lerner and colleagues demonstrated that passive transfer of circulating or kidney-bound antibodies from GP patients caused glomerulonephritis in recipient monkeys. This seminal observation represented the first evidence that an autoantibody *per se* can cause the autoimmune disease [4]. Subsequently the non-collagenous (NC1) domain of theα3 chain of collagen IV was identified as the autoantibody target [5–8], providing the foundation for the discovery of the α4, α5 and α6 chains and the emergence of collagen IV as a family of six αchains [9]. Immunization with recombinant NC1 domains revealed that the α3NC1 specifically induces severe proteinuria and glomerulonephritis in animal models [10–12,13•]. These findings fulfill criteria for Koch's postulates as applied to an autoimmune disorder, directly demonstrating a cause-effect relationship between a self antigen, the α3NC1 domain, and a pathogenic autoantibody in GP disease.

In the native form,α3 chain is an integral component of a collagen IV network, the principal part of the glomerular filtration barrier. This network is assembled by the selective association of the α3,α4 and α5 chains in a triple-helical protomer and by the oligomerization of protomers through end-to-end associations and intertwining of triple helices. Two protomers associate through carboxyl terminal domains forming α345NC1 hexamer, the GP autoantigen. Recent studies demonstrated that the α345 network is synthesized and deposited in the GBM exclusively by podocytes [14•]. Analysis of NC1 hexamers isolated from various basement membranes revealed the non-random association of individual α chains. The specificity of the networks assembly is governed by the NC1 domains [15–17].

In the current review we focus on recent advances in the characterization of circulating and kidney-bound autoantibodies, the architecture of GP autoantigen and epitopes. Several other aspects such as the role of cellular immunity or genetic predisposition in GP disease have been addressed in recent excellent reviews by other authors [18,19•,20•].

Heterogeneity of circulating and tissue-bound autoantibodies in GP disease

In GP disease, high level of serum creatinine (more than 5 mg/dL), crescent formation in more than 50% of the glomeruli, and dialysis dependence at the time of diagnosis are all associated with a poor kidney outcome. Analysis of sera from 79 GP patients in Sweden demonstrated that renal survival in patients who were not dialysis dependent at diagnosis was associated with lower levels of circulating anti-GBM antibodies [21]. In a recent retrospective study of 147 GP patients, the titer of circulating anti-GBM antibodies correlated with serum creatinine at diagnosis and had prognostic importance [22••]. Significant correlation observed between the avidity and the percentage of crescentic glomeruli in another study suggests that the avidity of circulating anti-GBM antibodies might play a role in the pathogenesis of anti-GBM disease [23].

Our recent studies show that the properties (NC1 and epitope specificity, and affinity) of circulating and tissue-bound autoantibodies are essentially identical [24••], suggesting that in GP disease only a fraction of antibodies is bound to the tissue target, the α345 collagen IV

network of kidney and lung. Comparison of autoantibody levels further implies that the production of pathogenic autoantibodies in GP disease greatly exceeds adsorptive capacity of GBM and indicates that the titer of circulating autoantibodies is a valid measure for the severity of disease.

In addition to circulating antibodies to the α3NC1 domain, lower binding to other NC1 domains of collagen IV (α1, α2, α4 and α5) has been reported [25–29] and interpreted as cross-reactivity. Recently, using recombinant NC1 domains of all six humanα-chains, we described distinct circulating antibodies specific for α5NC1 domain, which represent a second most abundant group of autoantibodies that occur in about 70% of GP patients [24••]. Elevated titers of circulating α5-GP antibodies are associated with unfavorable renal outcome. Furthermore, despite the highly variable reactivity of circulating antibodies to NC1 domains of collagen IV, only autoantibodies againstα3NC1 and α5NC1 domains are bound to basement membranes in kidney and lung of GP patients, indicating that both anti-α3NC1 and anti-α5NC1 antibodies contribute to the pathogenesis of GP disease. Thus, the α345NC1 hexamer is termed the GP autoantigen.

The α345NC1 hexamer is also the target for alloantibodies in patients with Alport post-transplantation nephritis (APTN). In Alport syndrome, mutations in any of three genes disrupt the expression and assembly of α345 collagen IV network [9], resulting in progressive glomerulonephritis and end-stage renal failure. About 5% of Alport patients develop APTN after kidney transplantation with subsequent allograft loss in the majority of cases [30]. APTN is mediated by the deposition of alloantibodies against α3NC1 and α5NC1 domains in response to the “foreign” α345 collagen IV network in the renal allograft [31–33].

Natural GP-like autoantibodies

Natural autoantibodies reactive with GBM were found and purified from normal human sera [34,35•], although at much lower titers and avidity compared to GP autoantibody. They belong to IgG class and bind theα3NC1, but not α1NC1 or α5NC1 domains. Most surprisingly, they recognize the same epitopes as autoantibody from GP patients [36]. Given the remarkable similarity of their properties to pathological GP autoantibody, it is unknown why natural antibodies do not elicit an autoimmune response. One major difference is the subclass restriction for natural anti-GBM antibodies to IgG2 and IgG4, while GP autoantibodies belong predominantly to IgG1 and IgG3, which led to the suggestion that altered subclass distribution of anti-GBM antibodies may be associated with development and progression of the GP disease [37•]. This is supported by association between levels (and avidity) of autoantibodies and severity of the GP disease [23]. In addition, GP patients with preserved renal function have autoantibodies restricted to the IgG4 subclass, in contrast to predominance of the IgG1 in GP group with severe renal damage [38]. The pathological role of various IgG subclasses is likely related to differential capacity for complement activation and selective binding to members of Fcγ receptor family, as has been recently demonstrated in mouse models of anti-GBM glomerulonephritis [39,40••].

In general, three major functions of natural antibodies have been proposed: host defense, housekeeping, and immune homeostasis. As a part of homeostatic function, natural antibodies might prevent the stimulation of autoreactive B cells either by masking autoantigen epitopes or through idiotypic regulation. Currently, there is no evidence for natural anti-GBM antibodies to operate thorough this mechanism. Further studies are required to elucidate the functional significance of natural anti-GBM antibodies in the establishment or maintenance of the immune tolerance in healthy humans, as well as any potential protective role in GP disease.

Three-dimensional structure of the native GP autoantigen

Understanding of the quaternary organization of the GP autoantigen, the α345NC1 hexamer, was greatly advanced by the determination of the crystal structure ofα121NC1 hexamer in 2002 by two independent groups [17,41]. Given the high degree of sequence conservation between NC1 domains (70–83% identity), a three-dimensional model forα345NC1 hexamer was built subsequently using homology modeling, molecular dynamics and energy minimization [42] (Fig.1). The NC1 hexamer is an ellipsoid-shaped structure composed of two identical trimeric caps formed by the carboxyl terminal NC1 domains of α345 protomers. The α3NC1, α4NC1 and α5NC1 domains in each trimeric cap are organized through unique domain swapping interactions, while the large planar interface between caps is stabilized by extensive hydrophobic and hydrophilic interactions. Distinct sites within each NC1 monomer govern the specificity of assembly of chain-specific hexamers (i.e., α121, α345, and α556 [16,43,44]. Confirmatory evidence for this assembly mechanism was recently obtained from in vivo studies, where substitution of mouse NC1 domain in α3 chain for human α5NC1 impaired the assembly of theα345 network in the GBM resulting in progressive loss of renal function [45•].

The structure of theα345NC1 hexamer is reinforced by novel sulfilimine bonds, which act as molecular fasteners that stabilize collagen IV network [42] (Fig. 1). This covalent bond (S=N) connects theε-N atom of hydroxylysine-211 (or lysine) and the S atom of methionine-93 of opposing NC1 monomers [46••]. In the GBM of human and primates, α345NC1 hexamers occur in crosslinked and uncrosslinked forms, indicating that the crosslinking event is incomplete [47], while in mice, only the crosslinked form occurs (*vide infra*).

Architecture and binding properties of GP epitopes

Understanding of the structure and binding properties of GP epitopes is of pivotal importance in deciphering clues about etiology and pathogenesis. Major advances have been made on this topic but key questions remain unanswered. NC1 monomers harbor the epitopes because they robustly bind GP antibodies. The crosslinked α345NC1 hexamer, the native GP autoantigen, is inert to antibody binding, but upon the perturbation of its quaternary structure, dissociated monomer and dimer subunits bind the GP antibodies. Thus, upon hexamer dissociation, the subunits undergo a structural transition with a concomitant display of pathogenic neoepitopes. The major epitopes in the α3NC1 subunit are encompassed by two major regions, designated E_A- α3 (residues 17–31) and E_B-α3 (residues 127–141) [48,49]. In the α5NC1 subunit the major epitope was recently mapped to a homologous region designated E_A-α5 [24••].

The complete identity and three-dimensional structure of epitopes in dissociated subunits are unknown. However, the structures of the critical E_A and E_B regions are known in the context of the inert native NC1 hexamer (Fig. 1). The E_A and E_B regions are located close to the triple helical junction and share significant structural similarity. This spatial arrangement sequesters several critical residues (V and L), those established by site-directed mutagenesis [50], at the interfaces between NC1 subunits. Such interactions can block antibody binding. Conformational changes likely occur upon hexamer dissociation which transition these regions together with other critical residues into a pathogenic conformation that confers antibody binding. Such conformational changes have been observed upon dissociation of α121NC1 hexamers using circular dichroism [43], and also suggested from the decreased binding of APTN alloantibodies to dissociated of NC1 hexamers from GBM [24••,32]. Additional support for the absence of the pathogenic epitopes within native α345NC1 hexamers has been provided in a recent study by Borza et al., who showed that GP

autoantibody failed to induce glomerular disease upon injection into *Fcgr2b*$^{-/-}$ mice, while the Alport alloantibody deposited along the GBM and elicited crescentic glomerulonephritis [51••].

Clinical relevance of GP epitopes

Of two major regions, E_A and E_B, which encompass epitopes for GP autoantibody in α3NC1 domain, only antibodies reactive to E_A correlated with renal outcome in one study [49]. Immunization with chimeric protein bearing the E_A region induced severe glomerulonephritis in rats with proteinuria and IgG deposits in GBM by 3 weeks, while chimeric protein containing the E_B region was not effective by itself, but enhanced the disease when administered in combination with E_A [52]. More recent analysis of a large number of GP patients showed elevated levels of both E_A and E_B reactive circulating antibodies without a change in E_A/E_B ratio in the dialysis-dependent versus dialysis-independent group of GP patients, suggesting that both regions are pathogenically relevant [22••]. Furthermore, kidney-bound GP autoantibodies react to both E_A and E_B regions of the α3NC1 monomer [24••]. In addition, the immunodominant epitope for circulating and kidney-boundα5-GP autoantibodies was identified within the α5NC1 monomer region homologous to the E_A region of α3NC1. Lower titers and affinity of anti-α5NC1 antibodies suggest that they develop secondary to anti-α3NC1 antibodies during progression of the GP disease. This is supported by the observation that in some GP patients, predominantly with preserved renal function, specificity of circulating antibody is restricted to the α3NC1 monomer, while none of GP sera had reactivity limited to α5NC1 [24••]. Dissociation of uncrosslinked α345NC1 hexamers by high affinity anti-α3NC1 autoantibodies [47] may lead to exposure of neoepitopes within α5NC1 subunit. Subsequent development of anti-α5NC1 antibodies may involve intermolecular epitope spreading, which has been demonstrated in the rat model of autoimmune glomerulonephritis [53].

Role of a structural transition in etiology of GP disease

Overall, recent findings support the contention that quaternary interactions together with sulfilimine crosslinks within α345NC1 hexamers provide a constraint against transition of the E_A and E_B regions into a pathogenic conformation. In basement membranes an additional constraint is provided by the triple helical domains tethered to the NC1 hexamer (Fig. 2). In the uncrosslinked hexamer (C-2), conformational constraint is diminished, and shifts equilibrium towards the trimers (C-3). Moreover, GP antibodies can induce a conformational change, dissociate conformer-3 and form antigen-antibody complexes, which is consistent with the binding to uncrosslinked hexamer *in vitro* [47] and passive transfer experiments [4,54]. In mouse models, passive transfer of GP antibodies does not induce glomerulonephritis because the hexamers in GBM are completely crosslinked (C-1) [51••].

We postulate that the initiation of GP disease involves conformational transition in crosslinked or uncrosslinked hexamers forming GP neoepitopes that elicit both antibody production and binding. This transition may be triggered by a single factor or combination of factors, such as post-translational modifications (nitrosylation, glycation), oxidation damage [55], or proteolytic cleavage. Indeed, formation of an alternative disulfide bond in α3NC1 results in a hexamer with enhanced autoantibody binding [56]. The role of conformational changes in the formation of the pathogenic neoepitopes is additionally supported by the ability of isolated NC1 dimers and monomers, or acid-dissociated, but not intact GBM hexamers, to induce glomerulonephritis in animal models [10,57]. Furthermore, environmental factors such as smoking or exposure to organic solvents could inhibit the putative enzyme that catalyzes formation of sulfilimine bonds thereby increasing the

proportion of uncrosslinked hexamers that are more susceptible to conformational transitions.

The requirement for hexamer transition into a pathogenic conformation is analogous to the hallmark features of “conformational diseases” such as Alzheimer and prion diseases. They arise when an extracellular protein undergoes a pathogenic conformational change or misfolding with resultant self-association into toxic aggregates deposited in tissues. In this context, GP disease could be classified as an autoimmune conformational disease, and its pathogenesis may involve an initiating event that perturbs the quaternary structure of the autoantigen and induces a pathogenic conformation, forming neoepitopes that become both the culprit and victim.

Conclusion

Recent evidence for a role of conformational transitions in the autoantigen in GP disease frames three fundamental questions for the future studies: 1) What is the triggering agent? 2) What is the conformation of the GP autoantigens (dissociated α3NC1 and α5NC1 monomer and dimer subunits) in the GBM? and 3) What is the there-dimensional structure of the complete GP epitopes in dissociated monomers? The answers will provide further insight into mechanisms that underlie etiology of the autoimmune injury and lead to designing of autoantibody decoys for future therapies of GP disease.

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Key points

- In addition to α3NC1 autoantibodies, distinct α5NC1 autoantibodies are present in circulation and in kidney/lung bound form in the majority of patients with Goodpasture's disease and associated with poor renal prognosis.
- Natural anti-GBM antibodies of restricted IgG subclasses are present in serum of healthy individuals and may play immune protective role.
- Novel protein crosslink, a sulfilimine bond, is discovered in NC1 hexamers of collagen IV, which represents a new structural mechanism for conferring immune privilege in GP autoantigen.
- Analysis of epitopes for pathogenic antibodies in two forms of glomerulonephritis, GP disease and Alport post-transplant nephritis, suggests a role for conformational transition in the autoantigen in etiology and pathogenesis of GP disease.

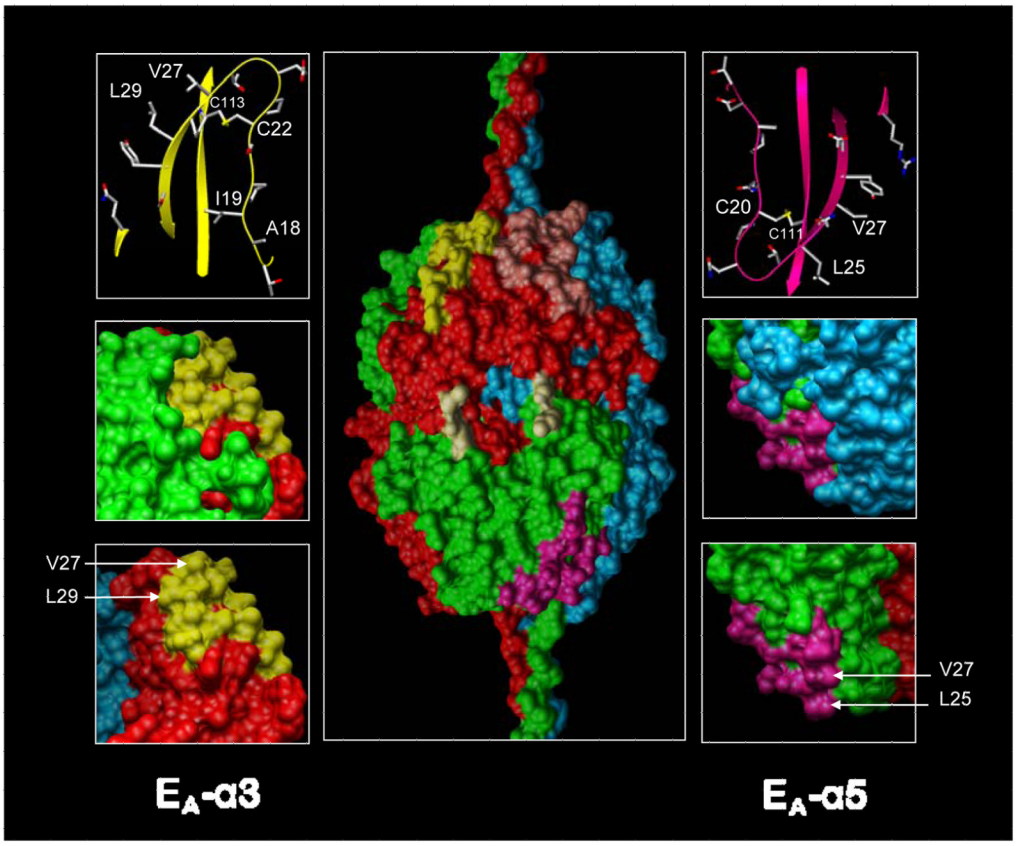

Figure 1. Three-dimensional structure of the GP autoantigen, α345NC1 hexamer of collagen IV

The hexamer is composed of two trimeric caps each consisting of α3 (*red*), α4 (*blue*) and α5 (*green*) NC1 monomers, and stabilized by sulfilimine bonds (*light yellow*) as shown in center. The model depicts location of the homologous regions E_A regions in α3NC1 (*yellow*) and inα5NC1 (*magenta*) domains, which become neoepitopes for GP autoantibodies upon hexamer dissociation concomitant with the exposure of residues and conformational change. The regions adopt a specific folding pattern of β-sheets stabilized by disulfide bonds as shown by ribbon diagrams (left and right top insets). Several critical residues are sequestered by lateral interactions with the α5NC1 and α4NC1 subunits in the α345NC1 hexamer as illustrated by removal of interacting NC1s (arrows on the bottom insets).

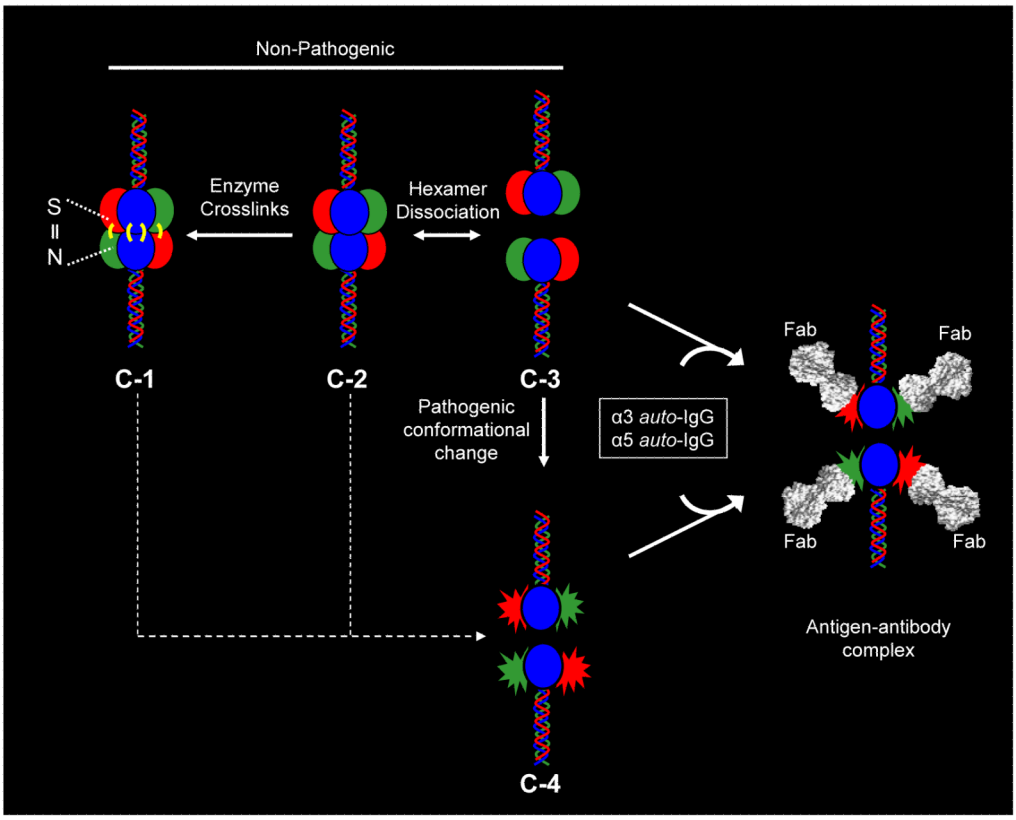

Figure 2. Potential role of conformational changes of α345NC1 hexamers in GP disease

The diagram represents a portion of the collagen IV network in which the α345NC1 hexamer is tethered to the triple-helical domain. Distinct conformational isoforms are shown: crosslinked form stabilized by sulfilimine bonds (C-1), non-crosslinked form (C-2), and form in which the NC1 hexamer is dissociated into trimers (C-3). In GP disease, C-3 may undergo a conformational change and exposure of critical residues resulting in the formation of neoepitopes in α3NC1 and α5NC1 subunits. This structural transition triggers formation of autoantibodies, which subsequently bind to C-3 and C-4. C-1 and C-2 could be potentially transformed into the pathogenic form C-4. GP autoantibodies in the immune complex are shown as F_{ab} fragments.