Published in final edited form as:

Matrix Biol. 2018 October ; 71-72: 240–249. doi:10.1016/j.matbio.2018.05.004.

Goodpasture's autoimmune disease – a collagen IV disorder

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Abstract

Goodpasture's (GP) disease is an autoimmune disorder characterized by the deposition of pathogenic autoantibodies in basement membranes of kidney and lung eliciting rapidly progressive glomerulonephritis and pulmonary hemorrhage. The principal autoantigen is the $\alpha345$ network of collagen IV, which expression is restricted to target tissues. Recent discoveries include a key role of chloride and bromide for network assembly, a novel posttranslational modification of the antigen, a sulfilimine bond that crosslinks the antigen, and the mechanistic role of HLA in genetic susceptibility and resistance to GP disease. These advances provide further insights into molecular mechanisms of initiation and progression of GP disease and serve as a basis for developing of novel diagnostic tools and therapies for treatment of Goodpasture's disease.

Keywords

Collagen IV; autoimmunity; Goodpasture's disease; autoantibodies; epitope; autoantigen; glomerular basement membrane; T cell

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V.P., A.R.K. and B.G.H. have no conflicts of interest to disclose.

Introduction

Over the last half a century of studies, Goodpasture's (GP) disease has emerged as a model disorder for exploring mechanisms that underlie autoimmunity. GP disease is an organ-specific autoimmune disorder characterized by linear deposits of autoantibodies along the glomerular basement membrane (GBM) (Fig.1), rapidly progressive glomerulonephritis, and often pulmonary hemorrhage. Pathogenic autoantibodies target specific type of collagen IV network classifying GP disease as an autoimmune collagen IV disorder. In the absence of pulmonary lesions, which occurred in about half of the patients, it is often defined as anti-GBM disease. GP is a rare disorder with the incidence of 1–2 cases per 1 million population per year [1, 2]. Without emergent treatment, it rapidly progresses to the end-stage renal failure with a permanent loss of kidney function and fatal outcome in about half of the patients [3, 4]. Early detection and combination treatment by the plasma exchange and immunosuppression to remove pathogenic autoantibodies significantly improve renal outcome and survival during last several decades [5–7]. GP disease affected both man and women with about equal frequency. The age distribution is bimodal with the first peak of occurrence during the third decade with a higher prevalence among men, and frequently observed pulmonary involvement. The second peak occurs around the age of 60 years, less frequently associated with pulmonary symptoms. Goodpasture's disease is a monophasic disorder with low recurrent rate [2, 8].

In a landmark study Lerner *et al.* showed that passive transfer of circulating or kidney-bound autoantibodies from GP patients to recipient monkeys produced severe glomerulonephritis, which provides the first evidence that antibody *per se* can cause the autoimmune disease [9]. Subsequent search for the antigen identified the non-collagenous (NC1) domain of a novel $\alpha 3$ chain of collagen IV as a target for the pathogenic autoantibody in the glomerular and alveolar basement membranes [10, 11]. Moreover, immunization with the recombinant $\alpha 3$ NC1 protein induced severe proteinuria and glomerulonephritis in animal models that closely resembled human GP disease [12, 13]. Collectively, these findings fulfill criteria for Koch's postulates as applied to an autoimmune disorder, demonstrating a direct cause-effect relationship between a self-antigen and a pathogenic autoantibody in GP disease.

Following studies led to the discovery of the $\alpha 4$, $\alpha 5$ and $\alpha 6$ chains and the emergence of collagen IV as a family of six α -chains ($\alpha 1$ – $\alpha 6$), which assembled in three distinct networks [14–16]. Collagen IV is a main constituent of all basement membranes, specialized form of extracellular matrix, which support tissue integrity and perform numerous key functions including cell signaling, morphogenesis, and tissue regeneration [17]. While Goodpasture's disease has been long recognized as an autoimmune collagen IV disorder, other basement membrane components are also targeted by antibodies in several autoimmune diseases [18].

Later studies identified distinct autoantibody targeting $\alpha 5$ NC1 domain in majority of GP patients and demonstrated their pathological relevance [19, 20]. This defined the $\alpha 345$ network of collagen IV as GP autoantigen (Fig.1), which expression is restricted to the basement membranes of kidney and lung underlying the pathogenesis of GP disease. Consequently, four pathogenic epitopes for GP autoantibodies were mapped within $\alpha 3$ and $\alpha 5$ NC1 domains [19, 21, 22].

Etiology of the GP disease remains largely unknown, but recent studies emphasized the role for conformational changes within $\alpha 345$ collagen IV network in eliciting an autoimmune response [19]. These conformational changes might result from aberrant posttranslational modifications of the autoantigen. In this respect, two enzymes were discovered recently, which might interact with Goodpasture autoantigen and potentially form a ternary complex in the GBM. Peroxidase, an extracellular heme peroxidase, catalyzes the formation of sulfilimine crosslinks in collagen IV, which confer structural reinforcement to collagen networks and immune privilege to Goodpasture autoantigen [23]. Consequently, dysregulation of peroxidase may perturb networks structure leading to the autoimmune disease. Second enzyme, Goodpasture binding protein (GPBP) is unusual serine protein kinase which phosphorylates human $\alpha 3$ NC1 domain [24]. GPBP is strongly expressed in human GBM, and its overexpression has been associated with an expanded and disorganized GBM in mice [25], suggesting that aberrant expression or activation of GPBP might result in increased phosphorylation of the autoantigen and initiate the autoimmune response in GP disease [24].

In the current review, we focus on recent advances in the characterization of pathogenic autoantibodies, architecture of the Goodpasture autoantigen and structure of the GP epitopes. We also summarized recent findings on the role of T cell tolerance in the context of genetic predisposition to GP disease. Several aspects such as diversity of clinical presentation, atypical forms of anti-GBM disease and advances in clinical management have been addressed in recent excellent reviews [26–28].

Pathogenic Goodpasture autoantibodies

In GP disease poor kidney outcome is commonly associated with high serum creatinine (over 5.7 mg/dL), large numbers of glomerular crescents (>50%) on renal biopsy, or a need for dialysis at presentation [5, 29]. Multiple studies demonstrated that severity of the disease also correlates with the level of circulating anti-GBM antibodies [19, 30, 31]. Low levels of circulating autoantibodies have been usually observed in rare cases of anti-GBM disease with normal kidney function [32]. There are also isolated reports of Goodpasture's disease negative for serum anti-GBM antibody, but with linear IgG deposition along the GBM proven by the immunofluorescence [33, 34], which might represent an early stage of GP disease, with treatment usually resulting in normalization of renal function.

Affinity of GP autoantibodies is another factor associated with crescent formation and glomerular damage [30, 35]. The average affinity of the purified Goodpasture antibody for the $\alpha 3$ NC1 domain is in 1–10 nM range [19, 21]. It has been found that the affinity of anti-GBM antibody is associated with the extent of kidney damage, with lower affinity of antibodies occurred in milder disease [35, 36].

The major class of Goodpasture autoantibody deposited along the GBM on kidney biopsies is IgG with exceptionally rare reports for IgA or IgM, which were classified as an atypical anti-GBM disease [37–39]. All four subclasses of IgG were detected in serum and kidneys [40–42], which together with the presence of both kappa and lambda light chains indicate that GP autoantibodies are polyclonal. Although the most common isotype of circulating and

kidney bound antibody belongs to IgG1, it was also shown that deposition of the IgG3 correlates with infiltration of inflammatory cells and renal damage [43]. Accordingly, IgG1 and IgG3 play a major role in the initiation and progression of the disease, which could be associated with their increased capability to activate complement and bind Fc γ receptors. This is supported by frequent linear or segmental C3 deposits within the GBM in GP disease. In contrast, patients with predominance of IgG4 autoantibodies have favorable renal outcome and may constitute a distinct subgroup of anti-GBM disease [36, 44, 45].

In addition to the ubiquitous autoantibodies against $\alpha 3$ NC1, distinct antibodies specific for the $\alpha 5$ NC1 domain were detected in serum of 70% of GP patients and associated with unfavorable renal outcome [19]. In addition, despite the frequent reactivity of circulating antibodies to other collagen IV NC1 domains, only autoantibodies against $\alpha 3$ and $\alpha 5$ NC1 domains are deposited in kidney and lung of GP patients, suggesting that both species might contribute to the pathogenesis of GP disease. Definitive evidence for the pathogenic role of $\alpha 5$ NC1 antibodies were provided by the recent unique case of GP patient with autoantibody restricted exclusively to the $\alpha 5$ NC1 domain, in the absence of $\alpha 3$ NC1 antibody [20]. Furthermore, immunization with recombinant $\alpha 5$ NC1 protein induced linear IgG deposits along GBM, kidney damage and alveolar hemorrhage in rats, mirroring pathogenic manifestations of the human GP disease and showing that the $\alpha 5$ NC1 domain *per se* causes the experimental GP disease. This suggests that development of pathogenic autoantibodies in GP disease could be initiated by dysregulated expression of $\alpha 3$ or $\alpha 5$ collagen IV chains, resulting in abnormal secretion of individual chains with exposed GP epitopes.

Goodpasture epitopes and structure of the autoantigen

Autoantibodies from GP patients were shown to bind to a 27 kDa protein from collagenase digest of the GBM, which was identified as the NC1 domain of $\alpha 3$ chain of collagen IV [11]. The identity of the GP autoantigen was confirmed by expression of the recombinant $\alpha 3$ chain and its NC1 fragment fully capable of binding GP antibody [46]. Besides the $\alpha 3$ NC1 domain, GP antibodies react with other NC1 domains, but with lower reactivity, which might be attributed to the cross-reactivity of $\alpha 3$ antibody, and unknown pathological relevance [47, 48]. Based on analysis of circulating and tissue-bound antibodies from more than fifty GP patients, we have uncovered that $\alpha 5$ NC1 monomer is a second target for autoantibodies [19].

Two conformational epitopes for GP autoantibodies designated E_A and E_B have been mapped within the $\alpha 3$ NC1 domain using $\alpha 1/\alpha 3$ NC1 chimeric proteins, whereby the non-immunoreactive $\alpha 1$ NC1 domain provided the scaffold into which short homologous $\alpha 3$ NC1 sequences were substituted to ensure correct folding. The E_A epitope has been localized in the amino terminal part (residues 17–31), while E_B epitope (residues 127–141) was identified in the central part of the $\alpha 3$ NC1 domain [21]. Four hydrophobic residues (Ala¹⁸, Ile¹⁹, Val²⁷, and Pro²⁸) together with distant Gln⁵⁷ are required for autoantibody binding to the E_A epitope [49, 50]. Autoantibodies specific for the E_A region are believed to play an important role in the pathogenesis of GP disease because they are the predominant in all sera (~60%) and have higher affinity for antigen [51]. Moreover, high titers of these antibodies are correlated with an unfavorable disease outcome [22]. The pathological role of

E_B epitope is more controversial. In animal model of autoimmune glomerulonephritis, immunization with chimeric protein bearing E_B region does not induce glomerulonephritis, but exacerbated the disease induced by the E_A containing chimera [52]. However, in GP patients comparable amounts of kidney bound antibodies specific for E_A and E_B epitopes of $\alpha 3$ NC1 have been found [19]. Elevated levels of circulating antibodies targeting both epitopes were detected in dialysis-dependent versus dialysis-independent GP patients [53]. In the recent study of over 100 GP patients, antibody targeting E_B epitope were identified as an independent risk factor for renal failure [54]. Thus, existing data suggest that both E_A and E_B epitopes are critical for pathogenesis of GP disease.

Further studies of $\alpha 5$ -GP autoantibodies using $\alpha 1/\alpha 5$ chimeras identified two distinct subpopulations which target two conformational epitopes in the $\alpha 5$ NC1 domain homologous to the E_A and E_B regions of $\alpha 3$ NC1 domain [19, 20]. Sequence analysis and molecular modeling highlight five residues that determine specificity of autoantibodies binding E_A and E_B epitopes of $\alpha 5$ NC1 over homologous regions in $\alpha 3$ NC1. Thus, four structurally homologous hotspots have been identified within NC1 domains of $\alpha 3$ and $\alpha 5$ chains of collagen IV as epitopes for autoantibodies in GP disease.

In the kidney glomerulus, the $\alpha 3$ and $\alpha 5$ chains are integral parts of the $\alpha 345$ collagen IV network of GBM (Fig.1C), which is expressed and deposited by podocytes and is a key component of the filtration barrier [55–57]. In this network, two triple-helical protomers associate via carboxy terminal part forming NC1 hexamer, which can be isolated from tissues by collagenase digestion [11, 58] (Fig.2). However, Goodpasture autoantibodies are minimally reactive with the native hexamer and the binding required dissociation of $\alpha 345$ hexamers into constituent subunits, suggesting that epitopes within hexamers are structurally sequestered [59]. This cryptic property of the autoantigen might represent a molecular mechanism underlying B cell tolerance in normal human population. It was initially suggested that immunodominant Goodpasture epitopes, E_A and E_B in $\alpha 3$ NC1 domain are sequestered within the $\alpha 345$ hexamer near the triple-helical junction by the neighboring $\alpha 5$ NC1 and $\alpha 4$ NC1 domains, respectively [60].

Unique cryptic property of GP autoantibodies binding to the $\alpha 345$ collagen IV network provided the framework for discovery a novel covalent crosslink, the sulfilimine bond ($-S=N-$) (Fig.2). This bond links together NC1 domains of two adjoining protomers, providing structural reinforcement to collagen IV networks [61, 62]. Subsequent studies addressed a key question of how this bond is formed resulting in the identification of peroxidasin [23], an extracellular peroxidase with unknown function originally discovered in *Drosophila* [63]. The identification of peroxidasin framed another important question about the chemical mechanism for bond formation, which brought up two surprising findings: peroxidasin produces hypobromous acid (HOBr) as a required intermediate for bond formation (Fig.2), and identification of bromine as an essential chemical element for the entire animal kingdom [64]. However, peroxidasin forms crosslinks only in assembled NC1 hexamer, while $\alpha 345$ collagen is synthesized intracellular as a trimer molecule, called protomer. The initiation and specificity of the collagen IV networks assembly is governed by the NC1 domains [65], however, molecular mechanism of this process remained unknown. Recently, we showed that formation of NC1 hexamers via head-to-head association of two protomers in the

extracellular compartment absolutely requires chloride ions identifying a critical step in the assembly of basement membrane which enable tissue architecture and function [66].

Intriguingly, sulfilimine crosslinks not only structurally stabilize NC1 hexamers, but also confer immune privilege to the $\alpha 3$ and $\alpha 5$ NC1 motifs that otherwise bind GP antibody [19, 67]. In contrast, the distinct population of uncrosslinked hexamers exist in GBM and can be dissociated by antibodies themselves upon binding to monomer subunits [68] (Fig.2). Furthermore, passive transfer of Goodpasture autoantibodies in mouse model failed to induce glomerulonephritis presumably due to the extensive degree of the $\alpha 345$ NC1 hexamer crosslinking in mouse GBM [69]. Thus, sulfilimine crosslinking of the collagen IV represents a novel mechanism for preventing autoantibody binding and subsequent tissue injury by posttranslational modification of an autoantigen.

The $\alpha 345$ network is also a target for antibody in another collagen IV related disease, Alport's post-transplantation glomerulonephritis (APTn), which occurs in 5% of patients with Alport's syndrome who receive kidney transplants [14]. Alport's syndrome is a genetic disorder caused by mutations in $\alpha 3$, $\alpha 4$ or $\alpha 5$ chains of collagen IV [70], which may result in absence of the $\alpha 345$ network in GBM. APTn is mediated by the deposition of alloantibodies in response to the "foreign" $\alpha 345$ collagen network of transplanted kidney that is absent in the kidneys of patients. Despite the common antigen target, APTn alloantibodies bind epitopes exposed on the native $\alpha 345$ NC1 hexamer, whereas Goodpasture autoantibodies require hexamer dissociation for unmasking of hidden epitopes [19, 71].

Goodpasture's disease, tolerance and HLA

Although uncommon, Goodpasture's disease is a prototypic autoimmune disease that has allowed us to not only learn about the structure of basement membranes and the nature of autoantibodies, but also more recently to dissect the fundamental role of HLA in the risk of protection from autoimmune disease [72, 73]. The classical $\alpha 3$ NC1-specific autoantibodies that bind to the glomerular and alveolar basement membranes are diagnostic [14]. Though antigen specific T cells are more difficult to detect, $\alpha 3$ NC1 specific CD4⁺ T helper cells of restricted clonality are present in GP disease patients [73].

While classical experiments published 50 years ago established the pathogenicity of anti-GBM antibodies [9], there is also substantial evidence that GP disease is mediated both by autoantibodies and T cells. CD4⁺ helper T cells are important in this disease in two ways. Most antibodies to protein antigens are T cell dependent, and the T follicular helper cell subset is likely to be essential to the production of anti-GBM antibodies [74]. CD4⁺ T cells specific for $\alpha 3$ NC1 are present both in normal humans and in GP patients [73, 75–77]. In addition to their essential role in the loss of B cell tolerance and the induction of autoantibodies, effector T cells are found in kidneys of patients with disease [78, 79]. Effector $\alpha 3$ NC1-specific CD4⁺ T cells can mediate disease in experimental anti-GBM glomerulonephritis [72, 73, 80] and B cell deficient animals develop glomerulonephritis when immunized with antigen [81, 82].

Further evidence for a critical role of CD4⁺ T cells in GPS comes from the strong HLA-DR associations in Goodpasture's disease. In humans, MHC II molecules that present antigenic peptides are represented by the HLA-DR, DP and DQ loci, and it is these HLA molecules that dictate how short linear peptides processed from proteins are presented to CD4⁺ helper cells. There are strong positive and negative HLA associations in GP disease. Several studies have consistently shown a strong association with HLA-DR15, with a relative risk of approximately 8.5 [83–86]. There are also HLA types negatively associated with the risk of disease, including HLA-DR1, HLA-DR7 and HLA-DR9 [84, 87]. At least in the case of HLA-DR1 and HLA-DR7, the HLA-DR15 mediated risk is abrogated by co-inheriting HLA-DR1 or DR7. Thus a permissive HLA is the “first hit” in the development of GP disease that sets the scene for the development of autoimmunity under unfavorable genetic and/or environmental conditions. While exact HLA allomorphs may differ, most autoimmune disease exhibit HLA risk alleles and protective alleles, and some feature HLA types that dominantly protect or modify the risk conferred by the risk allele [88, 89].

In addition to their roles in activating T cells in the periphery, HLA Class II molecules shape the CD4⁺ T cell repertoire in the thymus. In this process, central tolerance, T cell receptors (TCRs) that cannot recognize peptide/HLA complexes fail positive selection and these T cells die, while CD4⁺ T cells that recognize self-antigens with high affinity are deleted by negative selection, to lessen the risk of autoimmune disease (Fig 3A). However, some T cells with low affinity for self-antigens escape negative selection. Tolerogenic mechanisms operating in the periphery keep these cells in check in several ways including the generation of a further subset of CD4⁺ cells, thymically derived regulatory T cells (tTregs) that are thought to have intermediate affinity for self-peptides. These activated tTregs can suppress responses in both an antigen-specific and in more generalized manner.

The critical and immunodominant CD4⁺ T cell epitope of $\alpha 3$ NC1 is $\alpha 3_{135-145}$ [72, 73, 77]. The first study examining T cell epitopes in GPS showed that all humans responded to a peptide in this region [77]. A subsequent report showed that T cell reactivity to this area was present in a majority of patients [90]. HLA transgenic mice lacking all mouse MHC II but expressing HLA-DR15, DR1 or both DR15 and DR1 have a CD4⁺ T cell repertoire shaped only by the expression of these HLA allomorphs. Studies using these mice have confirmed the pivotal role of the $\alpha 3_{135-145}$ epitope [72, 73]. When immunized with recombinant mouse $\alpha 3$ NC1 or $\alpha 3_{136-146}$ (or human $\alpha 3_{135-145}$), HLA-DR15 transgenic mice are susceptible to autoimmune disease, including the development of anti-GBM antibodies. However, HLA-DR1⁺ and DR15⁺DR1⁺ transgenic mice are resistant to the development of autoimmunity and disease, mirroring the epidemiological findings in humans with the only difference in CD4⁺ T cell proinflammatory responses being to the $\alpha 3_{135-145}$.

The establishment of this system, together with the nature of GP disease with a clear set of diagnostic criteria, strong HLA associations and significant homology between the human and rodent $\alpha 3$ NC1 autoantigen means that GP can be used as a model to dissect the mechanism of HLA-mediated risk of and protection from autoimmune disease. The polymorphisms between the HLA-DRB1*15:01 and the HLA-DRB1*01:01 chains result in a marked difference in the structure of the HLA-DR15- $\alpha 3_{135-145}$ and HLA-DR1- $\alpha 3_{135-145}$ complexes (Fig 3B). The key $\alpha 3_{135-145}$ peptide bind in a different register to HLA-DR1,

resulting in a backbone flip and a significant dissimilarity between how this peptide is presented to T cells [73]. These structural features led to drastic functional consequences (Fig 3B–D). Both in naïve mice and in healthy humans, T cells that recognize the DR15- $\alpha 3_{135-145}$ complex are conventional T cells (Tconv) that have the capacity to be activated and differentiate into proinflammatory T helper Th1, Th17 or T follicular helper (TFH) cells, the CD4⁺ T cell subsets that are collectively critical to GP disease. In contrast to HLA-DR15, CD4⁺ T cells that recognize the DR1- $\alpha 3_{135-145}$ complex are largely anti-inflammatory thymically derived tTregs that maintain tolerance in both mice and humans. *In vitro* Treg depletion studies in mice and humans and *in vivo* studies in mice demonstrate that the reason people bearing both HLA-DR15 and HLA-DR1 are protected from disease is that HLA-DR1 aids in selecting $\alpha 3_{135-145}$ specific pro-tolerogenic Tregs [73].

While this is the first time in any autoimmune disease a mechanism for the protective effect of HLA molecules has been defined, peripheral tolerance comes in “layers”, and other mechanisms may also play important roles. Potential other mechanisms, which normally prevent loss of tolerance to $\alpha 3$ NC1, include T and B cell anergy, and T and B cell ignorance. In particular, there is an evidence for ignorance, with studies suggesting destructive processing of $\alpha 3$ NC1 domain in antigen presenting cells [91] and structurally cryptic $\alpha 3$ NC1 conformation B cell epitopes (E_A and E_B, as discussed above) suggesting that these critical epitopes may not be well displayed to the immune system, at least in some circumstances.

Etiology of the GP disease

Despite the significant progress achieved in characterization of the autoantigen and epitopes, the molecular mechanism of Goodpasture’s disease etiology remains unknown. It is generally assumed that pathogenesis of autoimmune disease involves environmental insult(s) in genetically predisposed individual resulting in miscommunication between the innate and adaptive immune systems, breakdown of tolerance, and recognition of self-antigens as the target of damaging immune response [92]. However, the nature of environmental triggers and the way of their interaction with genetic determinants of GP disease susceptibility is still obscure. The hypothesis of an environmental exposure as the initiating factor for GP disease received support from recent nation-wide study in Ireland, which demonstrated significant clustering of clinical cases in time and by region [2, 92]. Although initiating factors were not defined, potential triggers including cigarette smoking or inhalation of hydrocarbons may contribute to the development of lung hemorrhage through the local inflammation and perturbation of collagen IV network in the alveolar basement membrane resulting in epitope exposure and binding of antibody [93, 94]. In addition, viral or bacterial infections could play a role in the initiation of the disease via molecular mimicry of pathogen proteins or peptides. The first experimental evidence of potential molecular mimicry in anti-GBM disease was provided by demonstrating that microbial peptides mimicking T cell epitope of the $\alpha 3$ NC1 domain of collagen IV could induce glomerulonephritis and lung hemorrhage in WKY rats [95]. This notion is supported by the recent finding of antibodies in serum of GP patients reactive to microbial peptides that were similar to the human $\alpha 3$ NC1-derived peptide corresponding to the major linear B cell epitope [96, 97].

Treatment

Conventional treatment of GP disease includes combination of plasma exchange with immunosuppression to remove pathogenic autoantibodies results in non-selective removal of all serum proteins and possibility of adverse side effects. Therefore, alternative targeted therapies are highly desirable. Several reports showed that rituximab, chimeric antibody against human CD20 antigen expressed on B cells, is effective and could be used to decrease toxicity and side effects of cyclophosphamide and steroids in GP disease, although randomized control studies have not been performed [98–100]. Pathogenic autoantibodies could be also degraded by IdeS, a specific bacterial protease degrading IgG, which prevented antibody-mediated glomerulonephritis in mouse model for anti-GBM disease [101], representing another interesting option for the treatment of GP disease, a strategy that is now being pursued in a phase II human clinical trial (NCT03157037). Finally, immunoadsorption may be employed for selective removal of pathogenic autoantibodies, leaving other plasma components intact and avoiding the risk of infection or allergic reaction [102]. Remarkably, the first pediatric case of removal of IgG by immunoadsorption in GP patient has been recently reported with rapid improvement and normalization of kidney function [103]. Given that GP autoantigen is well characterized and could be produced in recombinant form, development of advanced immunoadsorption protocol selectively targeting pathogenic autoantibodies could be envisioned in a near future.

Concluding remarks

During the last 50 years, Goodpasture's disease has been a subject of extensive studies and can be now considered a prototypic autoimmune disease. The antigen is well defined with restricted number of epitopes, and α 345 collagen IV protomer has emerged as authentic autoantigen. Significant body of evidence was accumulated suggesting that the disease etiology involves both B- and T-cell mediated mechanisms.

The precise factors that act on a genetically susceptible background to induce disease remain unclear. Further insight into the mechanisms underlying the breakdown of immunological tolerance towards the autoantigen is required. This may ultimately enable a strategy to be developed whereby specific tolerance could be reestablished, and this could be applicable to a number of other autoimmune diseases.

Acknowledgements

The original research was supported in part by a grant R01 DK18381 from the National Institute of Diabetes and Digestive and Kidney Diseases to B.G.H.

Abbreviations used:

GBM	glomerular basement membrane
NC1	non-collagenous domain 1
GP	Goodpasture
HLA	human leukocyte antigen

MHC	major histocompatibility complex
Treg	regulatory T cells
Tconv	conventional T cells

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Figure 1.

A, Schematic diagram of the kidney glomerulus showing glomerular basement membrane (GBM), a key component of filtration barrier, as thin layer (blue ribbon) between endothelial cells (yellow) and podocytes (blue). **B**, Light and fluorescent microscopy of renal biopsy from Goodpasture's disease patient. Crescentic glomerulonephritis, a marker of severe glomerular injury, is made up of proliferating epithelial cells that line the Bowman's capsule, and infiltrating macrophages (top, Jones' silver stain). Immunofluorescent staining shows characteristic linear deposition of IgG autoantibody along the GBM (bottom). **C**, NC1 hexamer of $\alpha345$ collagen IV network in GBM is a target autoantigen for pathogenic Goodpasture autoantibody.

Figure 2.

Interaction of Goodpasture autoantigen, the α345 NC1 hexamer of collagen IV with autoantibodies. Space-filling structural model of the hexamer is composed of two trimeric caps each consisting of α3 (red), α4 (blue) and α5 (green) NC1 monomers. GP autoantibodies react with four epitopes of α3 and α5 NC1 monomers shown by different colors (light blue and orange in α3, yellow and blue in α5), with concomitant disruption of quaternary structure of uncrosslinked α345 NC1 hexamer. Part of hexamers stabilized by sulfilimine bonds (-S=N-, yellow) formed by peroxidase via production of hypobromous acid (HOBr). Crosslinked α345 NC1 hexamer is impenetrable to GP autoantibodies under native conditions, indicating that sulfilimine bond represents a novel molecular mechanism of immune privilege in GP autoantigen.

Figure 3.

A, CD4⁺ T cell tolerance in thymus: millions of unique T cell receptors are selected on the basis of HLA-peptide/TCR affinity. In negative selection the current paradigm is that high TCR affinity for HLA-self peptide complexes results in T cell deletion, low affinity results in egress of naïve T cells (some of which can become autoreactive) and intermediate-high peptide-HLA-TCR affinity results in activation and egress as Foxp3⁺ regulatory T cells (Tregs). **B**, The structure of the HLA-DR15 $\alpha 3_{135-145}$ complex favours selection of autoreactive conventional CD4⁺ T cells (Tconv). The 13 polymorphic amino acids in HLA-DR1B1*01 alter the HLA $\alpha 3_{135-145}$ structure so that selection of GP epitope-specific Tregs is favoured. **C**, HLA-DR15 selection sets the scene for the stripping away of tolerance in the periphery via other, as yet uncertain mechanisms with T cell activation and expansion into pathogenic T follicular helper cells (TFH) required for GP autoantibody formation and into helper Th1 and Th17 cells that effect glomerular injury. **D**, However, in individuals bearing both HLA-DR15 and HLA-DR1, potentially acting HLA-DR1 $\alpha 3_{135-145}$ specific Tregs dominantly suppress potential GP T cell activation.