Distinct Epitopes for Anti–Glomerular Basement Membrane Alport Alloantibodies and Goodpasture Autoantibodies within the Noncollagenous Domain of α3(IV) Collagen: A Janus-Faced Antigen

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Alport posttransplantation anti–glomerular basement membrane (GBM) nephritis is mediated by alloantibodies against the noncollagenous (NC1) domains of the α3α4α5(IV) collagen network, which is present in the GBM of the allograft but absent from Alport kidneys. The specificity of kidney-bound anti-GBM alloantibodies from a patient who had autosomal recessive Alport syndrome (ARAS) and developed posttransplantation nephritis was compared with that of Goodpasture autoantibodies from patients with autoimmune anti-GBM disease. Allograft-eluted alloantibodies reacted specifically with α3α4α5 NC1 hexamers, targeting their α3NC1 and α4NC1 subunits, and recognized a noncontiguous alloepitope formed jointly by the Eα and Eβ regions of α3NC1 domain. In contrast, human Goodpasture autoantibodies recognized the separate Eα and Eβ autoepitopes of α3NC1 but not the composite alloepitope. Molecular modeling of α3NC1 revealed that the alloepitope is more accessible within the NC1 hexamers than the partially sequestered Goodpasture autoepitopes. Overall, the specificity of alloantibodies indicated a selective lack of immune tolerance toward the α3 and α4(IV) collagen chains not expressed in patients with ARAS. Using COL4A3 knockout mice, a model of ARAS, it was shown further that acid-dissociated rather than hexamers, targeting their noncollagenous (NC1) domains to the adult Glomerular Basement Membrane (GBM), which is necessary for the long-term preservation of glomerular ultrafiltration function. Alport syndrome, the most frequent form of hereditary glomerulopathy, is caused by mutations in the genes encoding α3α5(IV) collagen chains, which prevent a developmental switch from the embryonic α1α2(IV) collagen network to the adult α3α4α5(IV) collagen in specialized basement membranes (1,2). The majority of Alport cases (≥85%) are X-linked (XLAS), as a result of mutations in the COL4A5 gene encoding α5(IV) collagen (3). A small proportion of patients develop autosomal recessive Alport syndrome (ARAS), as a result of mutations in both alleles of COL4A3 or COL4A4 genes (4,5). Over time, the Alport GBM undergoes characteristic ultrastructural changes, and patients develop hematuria and proteinuria and slowly progress toward ESRD, requiring dialysis or transplantation (6).

Among Alport patients that receive a kidney transplant, approximately 3 to 5% develop posttransplantation anti-GBM glomerulonephritis mediated by alloantibodies that bind to the allograft GBM but not to the Alport GBM (7,8). Alloantibodies were shown to react with the noncollagenous (NC1) domains of type IV collagen isoforms that are present in normal but not Alport kidneys (9). In general, patients with XLAS develop alloantibodies to the α5NC1 domain (10–12), and patients with ARAS develop alloantibodies against the α3NC1 domain (12,13). Both α3NC1 and α5NC1 occur natively as subunits of an (α3α4α5)2 NC1 hexamer (14), formed by the head-to-head association of two triple-helical α3α4α5(IV) collagen molecules in the GBM.

Alport posttransplantation anti-GBM nephritis in the renal allograft follows a similar clinical course as autoimmune anti-GBM disease, which is mediated by Goodpasture (GP) autoan-
tibodies that recognize the NC1 domain of the α3(IV) collagen chain (15). Together, these forms of anti-GBM disease provide a rare example of pathogenic autoantibodies and alloantibodies that target a common antigen. A detailed comparison between Alport alloantibodies and GP autoantibodies therefore could provide new insights into the molecular mechanisms underlying the cause and pathogenesis of anti-GBM disease, which may help to identify new therapies or preventive interventions.

The B cell epitopes targeted by GP autoantibodies have been characterized in detail (16–20), and two major conformational GP autoepitopes have been mapped to residues 17 to 31 (E1α) and 127 to 141 (E1β) of the α3NC1 domain (18,19). The location of alloepitopes within α3NC1 and α5NC1 has not yet been determined. Here, we characterized the specificity of anti-GBM alloantibodies eluted from the renal allograft of a patient who had ARAS and developed posttransplantation anti-GBM glomerulonephritis that ultimately led to the loss of allograft. ARAS alloantibodies specifically bound the α3NC1 and α4NC1 domains and recognized a noncontiguous α3NC1 alloepitope distinct from the GP autoepitopes. The alloantibody specificity indicated a selective lack of immune tolerance toward the α3 and α4(IV) collagen chains absent in ARAS. Using COL4A3/−/− mice, an animal model of ARAS (21,22), we further showed that the specificity of anti-GBM antibodies is critically influenced by the identity of the antigen used for immunization. Overall, the results suggest that distinct molecular forms of α3(IV) collagen cause ARAS posttransplantation anti-GBM glomerulonephritis and autoimmune anti-GBM disease.

Materials and Methods

Patients and Sera

A 28-yr-old Asian woman with ESRD secondary to ARAS underwent a kidney transplant at age 18, which failed 6 mo later as a result of reported occurrence of anti-GBM disease. In May 2002, the patient received a second cadaveric kidney transplant. Her immunosuppression regimen consisted of prednisone, mycophenolate mofetil, and tacrolimus. Initial renal function was excellent (serum creatinine 0.8 mg/dl) but worsened after 20 d (serum creatinine 1.5 mg/dl), and a percutaneous renal transplant biopsy was performed. The first renal biopsy contained 14 normal-appearing glomeruli. The interstitium showed a patchy lymphoplasmacytic infiltrate involving slightly less than 5% of the nonscarred parenchyma, with occasional PMN and eosinophils, with minimal tubulitis, and mild interstitial edema and hemorrhages. Arteries were unremarkable, without endothelialitis. Less than 5% of the tubulointerstitium was scarred. Immunofluorescence (IF) staining for IgG showed 1 to 2+ linear glomerular and focal linear tubular basement staining; C3 showed weaker (1+) staining with a pattern similar to IgG, as did κ and λ light chains (trace to 1+). Staining for IgA, IgM, C1q, fibrinogen, and albumin was negative or nonspecific. Electron microscopy showed only minimal effacement of foot processes and mild increase in mesangial matrix. A diagnosis of minimal infiltrate, not sufficient to diagnose acute rejection, was made, and anti-GBM staining was noted but without evidence of associated tissue injury.

The patient was treated with intravenous methylprednisolone sodium succinate and tacrolimus dose was increased, but serum creatinine persisted at 1.6 mg/dl. A second percutaneous transplant biopsy that was performed 1 wk later contained two glomeruli, one of which showed a small area of segmental necrosis with a small incipient cellular crescent (Figure 1A). The tubulointerstitium showed scattered lymphocytic infiltrate and mild edema. IF showed 2 to 3+ linear glomerular and focal 2+ tubular linear basement membrane staining for IgG (Figure 1B), κ and λ light chains, and a similar pattern for C3 (2+ in GBM; 1+ in tubular basement membranes). Other stains were negative or nonspecific. Electron microscopy studies were not performed. A diagnosis of anti-GBM antibody-mediated glomerulonephritis was made. Serum anti-GBM antibodies, initially below detection levels, became detectable but at low titer (1:32). Plasmapheresis was initiated, and mycophenolate mofetil was replaced with Cytoxan 2 mg/kg. Serum creatinine stabilized at a new baseline of 1.8 mg/dl until 3 mo posttransplantation. Serum creatinine rose to 2.9 mg/dl, and a third percutaneous renal biopsy was performed in September 2002. Thirteen of 17 glomeruli present showed global or near global sclerosis with extensive mostly fibrocellular and fibrous crescents and three cellular crescents, with fibrinoid necrosis in one of these glomeruli (Figure 1C). Interstitial fibrosis was approximately 50%, with propor-

![Figure 1. Histopathologic findings in the renal allograft biopsy of the patient with autosomal recessive Alport syndrome (ARAS). (A) Segmental necrosis and epithelial cell activation in a glomerulus (hematoxylin and eosin). (B) Linear glomerular basement membrane (GBM) staining with anti-IgG antibody (immunofluorescence). (C) Fibrocellular crescent and severe tubulointerstitial fibrosis (periodic acid-Schiff). Magnification, ×200.](image-url)
tional lymphocytic infiltrate without evidence of rejection. Vessels were unremarkable without endothelialitis. IF again showed strong linear glomerular and focal linear tubular basement staining for IgG (2 to 3+) and C3 (1 to 2+). Fibrinogen stained positively in crescents. Other stains were negative. A diagnosis of anti-GBM antibody-mediated glomerulonephritis with crescents and necrotizing lesions was made. While receiving plasmapheresis, the patient was admitted to the hospital with change in mental status, later diagnosed as West Nile virus encephalitis. Immunosuppression was withdrawn, and the patient died several months later. An autopsy was not performed.

Kidney-bound anti-GBM alloantibodies were eluted from the frozen portions of the three combined renal biopsies. Wet tissue was homogenized, washed with cold PBS, and eluted with 0.1 M glycine at pH 2.8 and then pH 2.2. The acid eluates were pooled, neutralized with 0.1 volumes of 1 M Tris buffer (pH 8.0), and analyzed by Western blot and indirect ELISA. Patient serum was not available for analysis. Sera or the first plasmapheresis fluid from patients with biopsy-proven autoimmune anti-GBM disease was used as a source of GP autoantibodies. Some of these sera were previously described (18), and additional GP sera were purchased from Wieslab AB (Lund, Sweden). All GP sera reacted specifically with r-α3NC1 monomers and α3NC1 chimeras that contained the E₆ and/or E₈ epitopes (see below).

Antigens
NC1 hexamers of collagen IV were collagenase-solubilized from human kidney cortex basement membranes and purified as described (23). Human GBM NC1 hexamers were fractionated into (α3α4α5)₂, (α1)₂/2/(α5)₂/6, and (α1α2α1)₃ NC1 hexamers by affinity chromatography on specific immobilized mAb, and their composition was verified by Western blot, as reported (14). Recombinant human α1-α5NC1 domains and chimeric α1/α3NC1 domains were expressed in human kidney 293 cells for correct folding and purified by affinity chromatography on immobilized anti-FLAG mAb (18). The chimeras consisted of an α1NC1 scaffold onto which the following α3NC1 residues were substituted: 17 to 31 (C2), 90 to 104 (C5), 127 to 141 (C6), 1 to 14 plus four amino-terminal Gly-X-Y triplets (C7), and 197 to 232 (C8). Composite chimera C26 contained both α3NC1 sequences found in chimeras C2 and C6. Mab3, a mouse IgG mAb specific for the four amino-terminal Gly-X-Y triplets (C7), and 197 to 232 (C8). ELISA Immunoassays
For indirect ELISA, Maxisorp microtiter plastic plates were coated overnight with purified antigens (250 ng/well) in carbonate buffer (pH 9.6) and blocked with 1% BSA. Immobilized proteins were incubated with diluted anti-GBM sera or eluted alloantibodies for 1 h, and IgG binding was detected with alkaline phosphatase–conjugated secondary antibodies followed by chromogenic substrate. The absorbance at 405 nm was measured with a Spectramax 190 ELISA plate reader (Molecular Devices, Sunnyvale, CA). The graphs show the mean absorbance and SD of three measurements. Comparisons between groups were performed by t test. P < 0.05 were considered significant. For inhibition ELISA, diluted GP sera or anti-α3NC1 mAb were preincubated for 2 h at room temperature with soluble antigens before assaying binding to immobilized r-α3NC1 in duplicate samples.

Western Blot
Purified human GBM hexamers (400 ng/lane) were separated by SDS-PAGE in 6 to 20% gradient gels and transferred to Immobilon P. Membranes were blocked with 5% casein, incubated overnight with eluate or control sera diluted in incubation buffer, then alkaline phos-
Anti-α3(IV)NC1 Alloantibodies Recognize a Noncontiguous Epitope

The finding of alloantibodies reactive with human α3NC1 but not α1NC1 monomers provided a direct strategy to map the α3NC1 alloepitopes using chimeric α1/α3NC1 constructs. Chimeras that contain short regions of α3NC1 substituted onto an α1NC1 scaffold for correct folding of conformational epitopes have been previously used to map the α3NC1 autoepitopes of GP autoantibodies (18,19). By indirect ELISA using a panel of α1/α3 chimeras (Figure 3), alloantibodies reacted only with a composite chimera, C26, which contained two noncontiguous α3NC1 sequences, E₅ and E₆. Because alloantibodies did not bind significantly to the C2 and C6 chimeras that contained the isolated E₅ and E₆ sequences, both regions must jointly form a composite α3NC1 alloepitope. Importantly, the same epitope specificity has been previously found for Mab3 (19), a well-characterized mouse anti-α3NC1 mAb produced against NC1 hexamers from bovine GBM (27).

GP Autoantibodies and ARAS Alloantibodies Target Distinct α3NC1 Epitopes

Remarkably, each of the two α3NC1 sequences harbored by the C26 chimera (E₅ and E₆) encompasses separate B cell epitopes, recognized by human GPₐ, GP₈, and GPAB autoantibodies (19). ARAS alloantibodies were distinguished from GPₐ autoantibodies by their lack of reactivity toward C2 and C6 chimeras that contained individual autoepitopes (see Table 1). ARAS alloantibodies were further distinguished from GPₐ autoantibodies because the latter did not react with C26 chimera. However, previous studies of the epitope specificity of human GP antibodies have not entirely ruled out the possibility that autoimmune GP sera may contain autoantibodies against the composite α3NC1 alloepitope, E₅&E₆. Because in standard immunoassays the putative GP reactivity against the composite α3NC1 alloepitope would be masked by the binding of GPₐ and GP₈ autoantibodies to the individual E₅ and E₆ epitopes, a different strategy was necessary to address this possibility.

The presence of GP autoantibodies against a composite E₅&E₆ epitope was assayed by inhibition ELISA, comparing antibody binding with immobilized α3NC1 in the presence of soluble chimeras. For comparison, Mab3, which targets a composite α3NC1 epitope similar or identical to that of ARAS alloantibodies, was inhibited only by the composite chimera C26 but not by the combination of single chimeras C2 and C6 (Figure 4A). In contrast, the inhibition of human GP autoantibodies by the C26 chimera was indistinguishable from the combined effect of C2 and C6 chimeras (Figure 4B). These results were confirmed using GP sera from 11 patients (Figure 5). Thus, GP sera do not contain autoantibodies against the composite α3NC1 alloepitope E₅&E₆. Overall, the results indicate that human Alport alloantibodies and GP autoantibodies have distinct specificities, despite targeting epitopes within a narrow region of α3(IV) collagen.

Distinct Locations of α3NC1 Alloepitopes and Autoepitopes

Even though the E₅ and E₆ regions are separated by approximately 100 residues in the primary structure of α3NC1, they are adjacent in the tertiary structure, as shown by a structural model of the α3NC1 domain build based on its homology to α1NC1 domain within the crystal structure of the [α₁α₃]₂2₃ hexamer (Figure 6). Therefore, the noncontiguous epitopes of ARAS alloantibodies and Mab3 must include residues from both E₅ and E₆ regions that are located in close proximity to each other. An analysis of the α3NC1 model revealed that residues 20 to 26 of the E₅ region and 137 to 141 of the E₆ region were within <10 Å of each other. Moreover, these residues
mapped to an accessible location on the surface of the NC1 hexamer, and none was in close proximity (\( \leq 10 \) Å) to the flanking NC1 domains in the hexamer complex (Figure 6). The accessibility of the composite epitope is further supported by Mab3 binding to native \( \alpha_{3}NC1 \) holo-hexamers (23), similar to XLAS alloantibodies (19,28). In contrast, the epitopes of all GP antibody subsets are cryptic and inaccessible for autoantibody binding unless the hexamer is dissociated (19,29). The most likely explanation for the crypticity of GP epitopes is steric hindrance of key epitope residues by the flanking \( \alpha_{4}NC1 \) and \( \alpha_{5}NC1 \) domains (14,29). Indeed, among the four EA residues identified in the epitope of immunodominant GPA antibodies (30), none is proximal to the EB region, whereas two are adjacent (within 10 Å) to the \( \alpha_{5}NC1 \) domain (Figure 6). Overall, the molecular architecture of the \( \alpha_{3}NC1 \) epitopes suggests that exposed epitopes within the native NC1 hexamers are alloantigenic, being targeted by alloantibodies in posttransplantation anti-GBM nephritis, whereas cryptic epitopes within isolated \( \alpha_{3}NC1 \) subunits are autoantigenic, being targeted by autoantibodies in autoimmune anti-GBM disease.

**Figure 6.** Analysis of GP sera by inhibition ELISA. Eleven GP sera were incubated with soluble \( \alpha_{3}NC1 \) or chimeras (10 μg/ml), and then binding to immobilized \( \alpha_{3}NC1 \) monomers was measured by ELISA. The graph shows means and SEM. Inhibition of GP sera by chimera C26 was not significantly different from that produced by a combination of C2 and C6 chimeras (\( P = 0.598; \) paired t test).

Differential Molecular Forms of \( \alpha_{3}NC1 \) Antigen Elicit Anti-GBM Antibodies with Distinct Specificities in COL4A3\( ^{−/−} \) Alport Mice

To gain further insight into the nature of the antigen that triggers production of posttransplantation anti-GBM alloan-

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\(^a\) GP, Goodpasture; ARAS, autosomal recessive Alport syndrome; NC1, noncollagenous; ND, not determined. The properties of the ARAS alloantibody characterized in this work are contrasted to those of the four subpopulations of GP autoantibodies previously described (19). ARAS alloantibodies differ from all other GP autoantibody subsets but have similar properties to Mab3. It is important to note that GP\(_{AB}\) autoantibodies do not target a composite \( E_{A&B} \) epitope but rather cross-react with both \( E_{A} \) and \( E_{B} \) epitopes, which have 47% sequence identity (19).

\(^b\) Reduction or treatment with 6 M guanidinium of human glomerular basement membrane hexamers strongly reduced binding of ARAS alloantibodies, relative to untreated hexamers, suggesting that the ARAS alloepitopes are conformational and accessible in the native NC1 hexamers. The experiment was performed only once.

**Table 1.** Comparison of human anti-\( \alpha_{3}NC1 \) GP autoantibodies and ARAS alloantibody\(^a\)

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**Figure 4.** Inhibition ELISA analysis of the specificity of anti-\( \alpha_{3}NC1 \) antibodies. Binding of Mab3 (A) or GP antibodies (B) to immobilized r-\( \alpha_{3}NC1 \) monomers (50 ng/well) was inhibited using various concentrations of soluble r-\( \alpha_{3}NC1 \) (\( \bullet \)) and chimeras C2 (\( \square \)), C6 (\( \triangle \)), a mixture of equal amounts of C2 and C6 (\( \bigcirc \)), and C26 (\( \bigcirc \)).
tibodies, we used a mouse model of ARAS (22). We hypothesized that native NC1 hexamers would elicit mouse anti-GBM antibodies similar to human ARAS alloantibodies, whereas isolated NC1 subunits would induce mouse anti-GBM antibodies similar to human GP autoantibodies.

**COL4A3**/H11002/H11002 mice were immunized with native and acid-dissociated NC1 hexamers that were isolated from wild-type mouse GBM, as well as with r-**COL4A3**/H9251NC1 monomers. All antigens elicited mouse IgG antibodies that reacted strongly with murine **COL4A3**/H9251NC1 hexamers but were distinguished by their reactivity toward **COL4A3**/H9251NC1 monomers (Figure 7A). Mice that were immunized with r-**COL4A3**/H9251NC1 monomers produced the highest titers of anti-**COL4A3**/H9251NC1 antibodies. These antibodies reacted mainly with epitopes in the C2 and C6 chimeras (Figure 7B), therefore recapitulating the specificity of human GP autoantibodies. Surprisingly, murine antibodies to native hexamers had very low reactivity toward all NC1 monomers; some sera exhibited weak binding to **COL4A3**/H9251NC1 but not significantly higher than binding to α1NC1 monomers (thereby precluding epitope mapping). Antibodies in this group were least similar to human anti-GBM antibodies. It is interesting that mice that were immunized with acid-dissociated hexamers did produce antibodies that selectively targeted **COL4A3**/H9251NC1 monomers (Figure 7A), even though the immunogen comprised a mixture of α1-α5 NC1 domains that contained only approximately 10% α3NC1 (data not shown); this specificity most likely reflects the lack of immune tolerance toward α3(IV) collagen in COL4A3−/− mice, as noted for human ARAS. However, unlike murine antibodies to α3NC1 monomers, anti-α3NC1 antibodies from mice that were immunized with dissociated hexamers did not react with the C2 and C6 chimeras (Figure 7B), being similar in this respect to human ARAS alloantibodies. These results demonstrate that anti-GBM antibodies with distinct epitope specificities are induced by different molecular forms of the α3NC1 antigen.
Discussion

GP autoimmune disease and Alport posttransplantation anti-GBM nephritis are distinct forms of anti-GBM antibody disease that implicate a common antigen, the NC1 domains of the $\alpha 3\epsilon 4\alpha 5$(IV) collagen network. This provides a rare opportunity to establish whether differences in the immune setting (normal expression of autoantigen versus its absence in genetic disease) can shape the fine specificity of human anti-GBM antibodies. GP autoantibodies bind to conformational autoepitopes within the $\alpha 3$NC1 domain, previously identified using chimeric $\alpha 1/\alpha 3$ NC1 domains. In this study, using the same chimeric constructs, we established for the first time the epitope specificity of anti-$\alpha 3$NC1 autoantibodies, which were eluted from the renal allograft of one patient who had ARAS and developed severe posttransplantation anti-GBM antibody nephritis. We show that ARAS alloantibodies and GP autoantibodies bind distinct alloepitopes and autoepitopes within $\alpha 3$NC1. For comparison, inhibitory anti-factor VIII autoantibodies and autoantibodies from patients with congenital or acquired hemophilia A both target common immunodominant epitopes within the A2 and C2 domains of factor VIII (31,32). Hence, $\alpha 3$(IV) collagen is the first human pathogenic antigen shown to harbor distinct alloepitopes and autoepitopes—thus, a “Janus-faced” antigen.

The chain and epitope specificity of kidney-eluted ARAS alloantibodies characterized in this work suggests a selective lack of immune tolerance toward collagen IV chains and networks that are not expressed in patients with ARAS. The alloantibodies reacted specifically with the NC1 hexamers of the $\alpha 3\epsilon 4\alpha 5$(IV) collagen network, which is absent in Alport syndrome, but not with NC1 hexamers derived from the $\alpha 1\alpha 2$(IV) or $\alpha 1\alpha 2/\alpha 5\alpha 6$(IV) networks, normally expressed in ARAS (33,34). The major target of kidney-bound ARAS alloantibodies is the $\alpha 3$NC1 subunit of the $\alpha 3\epsilon 4\alpha 5$NC1 hexamer. This is not unexpected, because anti-$\alpha 3$NC1 antibodies have been repeatedly found in the sera of patients with ARAS posttransplantation nephritis (12,13,35). The $\alpha 4$NC1 domain is a novel, albeit minor, target of ARAS alloantibodies. Anti-$\alpha 4$NC1 alloantibodies have been previously described in the sera of three patients with XLAS and posttransplantation nephritis (35). Lack of reactivity toward the $\alpha 5$NC1 subunit suggests that central and/or peripheral immune tolerance toward the $\alpha 5$(IV) collagen may be established in ARAS because this chain remains part of the unaffected $\alpha 1\alpha 2/\alpha 5\alpha 6$(IV) collagen network.

The presence or absence of immune self-tolerance toward $\alpha 3$(IV) collagen further affects the epitope specificity of anti-GBM antibodies. GP autoantibodies preferentially target $\alpha 3$NC1 autoepitopes that are structurally sequestered within the native NC1 hexamers but not exposed $\alpha 3$NC1 epitopes (19). Therefore, an autoimmune response may be triggered upon unmasking of cryptic neo-epitopes by putative pathogenic factors that affect the hexamer structure, which provide a mechanism for circumventing the normal self-tolerance toward native antigen. In contrast, in ARAS, lack of tolerance toward the $\alpha 3$(IV) and $\alpha 4$(IV) collagen chains would allow production of alloantibodies against exposed $\alpha 3$NC1 alloepitopes.

Characterization of anti-GBM antibodies produced in COL4A3−/− mice suggests that the molecular form of the $\alpha 3$NC1 antigen is an additional factor modulating the specificity of anti-GBM antibodies. Only $\alpha 3$NC1 monomers elicited murine antibodies with similar specificity to human GP autoantibodies, thus implicating $\alpha 3$NC1 monomers as the antigen initiating autoimmune anti-GBM disease. Consistent with this paradigm, GP antibodies were shown to target preferentially a subset of $\alpha 3\epsilon 4\alpha 5$ NC1 hexamers composed of monomer subunits only, inducing hexamer dissociation upon binding (29). Surprising, immunization with acid-dissociated rather than native NC1 hexamers induced production of mouse autoantibodies most closely resembling human ARAS alloantibodies and Mab3. This unexpected finding suggests that molecular damage to $\alpha 3\epsilon 4\alpha 5$(IV) NC1 hexamers in the allograft GBM could be an important factor that triggers Alport posttransplantation anti-GBM glomerulonephritis.

ARAS alloantibodies that are characterized in this work were likely pathogenic, because they were eluted from a renal allograft with characteristic crescentic injury. However, patients who have Alport syndrome and receive a transplant may often exhibit linear IgG deposition in the allograft GBM (35–38) and/or circulating anti-GBM alloantibodies (35) in the absence of nephritis. Although nonpathogenic alloantibodies were not available for this study, further investigations comparing the properties of nephritogenic and non-nephritogenic Alport anti-GBM alloantibodies may provide a better understanding of the triggers and mechanisms that cause allograft nephritis in a small proportion of Alport transplant recipients. The findings presented herein represent the analysis of allograft-bound alloantibodies from a single patient with ARAS and posttransplantation anti-GBM glomerulonephritis. Because results may vary among different patients, future studies of circulating and kidney-bound alloantibodies from other patients with Alport posttransplantation nephritis are necessary to establish definitively the repertoire of specificities for anti-GBM alloantibodies.

A detailed knowledge of the collagen IV chains and epitopes that are targeted by pathogenic anti-GBM alloantibodies may lead to the development of preemptive therapeutic strategies aimed specifically at inducing immune tolerance in patients with Alport syndrome before a renal transplant. In rat and mouse models of GP disease, oral administration of autoantigen before immunization blunts the severity of anti-GBM nephritis by reducing the Th1 response (39,40). Such proactive intervention may be beneficial in the subgroup of patients who have Alport syndrome and are at risk for developing posttransplantation nephritis. Even though this complication affects a small proportion of Alport transplant recipients, its incidence in this group far exceeds that of autoimmune anti-GBM disease in the general population (estimated at 0.5 to 1 new cases per 1,000,000 people per year). Future studies in animal models of Alport syndrome will be instrumental for understanding how immune tolerance to $\alpha 3\epsilon 4\alpha 5$(IV) collagen is established and broken and how it can be modulated for therapeutic purposes.

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