A COL4A3 gene mutation and post-transplant anti-α3(IV) collagen alloantibodies in Alport syndrome


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A COL4A3 gene mutation and post-transplant anti-α3(IV) collagen alloantibodies in Alport syndrome. The X-linked Alport syndrome is associated with mutations and deletions in the COL4A3 gene, one of six genes which constitute the α-chains of type IV collagen in basement membranes. The autosomal recessive form of Alport syndrome is characterized by mutations and deletions in the COL4A3 and COL4A4 genes. A fraction of Alport patients who undergo renal transplantation develop anti-glomerular basement membrane (GBM) nephritis, which results in loss of the renal allograft function. Recently, the target for alloantibodies from an X-linked Alport patient with complete COL4A5 gene deletion was determined to be the α3 chain of type IV collagen. The present study characterized the post-transplant alloantibodies from an autosomal recessive Alport patient with anti-GBM glomerulonephritis and a COL4A3 gene mutation which predicted a loss of 85% of the α3(IV) NCI domain. The specificity of these new antibodies were studied using glomerular GBM antigens and analytic assays. The results establish the target for the alloantibodies from an autosomal recessive Alport patient with COL4A3 deletion as principally the α5(IV) collagen chain, similar to the post-transplant alloantibodies from X-linked Alport patients with COL4A5 gene deletions. The absence of α3(IV) chain in the GBM of patients with both these forms of Alport syndrome, due either to a failure of synthesis or a failure of assembly, presumably leads to a loss of immunologic tolerance for the α3(IV) NCI domain in transplanted allografts.

Alport syndrome is a progressive hereditary kidney disease characterized by hematuria, sensorineural hearing loss, and ocular lesions with structural defects in GBM [1-3]. The disease is primarily X chromosome-linked, but autosomal forms of inheritance are also known [4]. The X-linked syndrome is associated with mutations and deletions in COL4A5 gene, which encodes the α5(IV) chain, one of six genetically distinct type IV collagen gene products [3, 5-7]. The rare autosomal forms of Alport syndrome are associated with recessive mutations in the COL4A3 and COL4A4 genes which encode the α3(IV) and α4(IV) chains, respectively [8, 9].

Anti-GBM disease appears in 5 to 10% of Alport patients who receive a kidney transplant following the development of renal failure. In the recent years, insight into the structural nature of the defect in Alport GBM has been determined by establishing the target of anti-GBM alloantibodies produced by these patients [10-14]. Alloantibodies from seven post-transplant Alport patients, for example, were found reactive to the NCI domain of type IV collagen [9-14]. Three of these antisera, under closer scrutiny, did not bind to Alport GBM and were found reactive to the α3(IV) chain [11]. Recently, post-transplant anti-GBM alloantibodies harvested from a X-linked Alport patient with complete COL4A5 gene deletion was characterized [15]. These alloantibodies were also specifically targeted to the α3(IV) chain [15]. This study suggested a pivotal role for the α5(IV) chain in the secretion or assembly of type IV collagen co-expressing the α3(IV) moiety.

The present study characterizes the target antigen for the post-transplant alloantibodies from an autosomal recessive Alport patient with COL4A3 gene deletion.

Methods

Patient history

The case history of family VB is described elsewhere [8, 9]. The affected female had hematuria from age 4, and typical ultrastructural lesions of Alport syndrome on electron microscopy of a renal biopsy and sensorineural deafness. Renal function deteriorated gradually until hemodialysis was started at age 9. She received a renal allograft at age 10 and developed anti-GBM nephritis six months later. Her brother has hematuria, deafness, and deteriorating renal function. The parents have no hematuria, proteinuria or deafness. There is no known consanguinity, but the parents and their known ancestors originate from the same small village in the Netherlands. The affected female in family VB has a deletion of last 198 amino acids of the α3(IV) chain. This was predicted to result in a chain termination after 33 amino acids of the NCI domain.

GBM antigens and analytic assays

The preparation of the GBM constituents, fibronectin, laminin, heparan sulfate proteoglycan, entactin, 7 S domain of type IV collagen, pepsin solubilized triple helical fragments of type IV collagen and NCI domains of type IV collagen α-chains was described previously [16]. Recombinant human type IV collagen α-chains (α1-α5) were expressed in E. coli and purified as before [17].
SDS-PAGE in one and two dimensions was carried out with 10 to 20% linear gradient gels and the discontinuous buffer system of Laemmli [18]. Electrophoresis in the first dimension of the two-dimensional electrophoresis was performed according to Langeveld et al [19]. Electrophoresis in the second dimension was performed according to Timoneda et al [20].

Immunofluorescence [11]. Western blotting [16, 21], and direct enzyme-linked immunosorbent assays (ELISA) were performed as previously described for this laboratory [22]. Inhibition ELISA was performed as previously described [23]. The dilutions for the α-chain specific antibodies were 1:50, a saturating antibody concentration for the binding of the bovine NC1 hexamer. The anti-α1(IV) to α5(IV) chain specific antibodies have been previously described [16]. The α6(IV) chain specific antibody was made recently to a conserved 12 amino acid peptide of the NC1 domain. (R. Kalluri, J. Zhou, and B.G. Hudson, unpublished data).

**Results**

**Localization of anti-GBM alloantibodies in renal tissues**

Circulating post-transplant anti-GBM alloantibodies from the Alport patient was evaluated for its capacity to bind the GBM of the Alport kidney of the same patient by indirect immunofluorescence. The alloantibodies did not bind to the Alport GBM, suggesting a lack of the certain GBM antigen(s) which are otherwise present in the transplanted kidney (Fig. 1A). The transplanted kidney showed endogenous IgG binding to the GBM and TBM, and the alloantibody binding was further enhanced.
upon incubation with the circulating alloantibodies (Fig. 1B). These results suggest that additional binding sites for the alloantibodies in the transplanted kidney are accessible in vitro compared to in vivo, and that there is a structural difference within the GBM of the Alport and transplanted kidney.

**Specificity of alloantibodies to GBM constituents and bovine type IV collagen domains**

The specificity of post-transplant anti-GBM alloantibodies from an autosomal recessive Alport patient with COL4A3 deletion was determined using bovine GBM constituents and bovine NCI domains of the α-chains of type IV collagen. The GBM constituents used were: fibronectin, laminin, HSP, heparan sulfate proteoglycan, E, entactin, 7 S, 7 S domain of type IV collagen, pepsin solubilized TH, triple helical fragments of type IV collagen and dimers and monomers of NCI domains of type IV collagen α-chains. The dilution of alloantibodies was 1:500. The control serum did not bind to any of the GBM constituents.

**Discussion**

In several previous studies, the target for X-linked Alport alloantibodies was identified as the NCI domain of type IV collagen [10–14]. Hudson et al have shown the target for three post-transplant alloantibodies as the α3(IV) chain of type IV collagen [11]. Kleppel et al implicated the α5(IV) chain as a target for alloantibodies in their patients [25, 26]. Their assumption of α5 chain reactivity was
Identification of the bovine NC1 domains that bind the Alport alloantibodies. Bovine NC1 hexamer was prepared as previously described [16]. The two dimensional electrophoresis and immunoblotting was used to analyze the binding NC1 hexamer with Alport alloantibodies. The spots that reacted with the alloantibodies are 16, 17, 18, 20, 22 and 23. All these spots have been previously identified by chain specific antibodies and N-terminal amino acid sequencing (identical spots analyzed from the α3(IV) NC1 dimer pool) as the α3(IV) dimers and monomers [16]. The dimers and monomers of NC1 domain are shown as D and M, respectively. The molecular weight of dimers (D) and monomers (M) was around 54 kDa and 28 kDa, respectively. The pI range (6 to 8) is shown at the bottom. This figure can be compared with the reactive spots seen in the Fig. 3, panels F, G and H in the manuscript by Gunwar et al [16].

Inhibition ELISA with type IV collagen α-chain specific antibodies. A. A dilution curve of Alport alloantibodies binding to the NC1 hexamer as analyzed by direct ELISA. B. The plates were coated with 200 ng of NC1 hexamer after boiling for 10 minutes in the presence of 6 M guanidene HCl, 50 mM tris-cl pH 7.5. The NC1 hexamer was allowed to bind to one of the six different α-chain specific antibodies ([()] α1 + α2; (■) α3; (○) α4; (△) α5; (▲) α6), upon which the NC1 hexamer was allowed to bind with Alport alloantibodies. The binding of the Alport alloantibodies was significantly inhibited by anti-α3(IV) antibodies.

Based on two lines of evidence; the alloantibodies reacted to a 26 kDa band in the immunoblotting studies with NC1 hexamer [25], and a monoclonal antibody which binds a 26 kDa band in NC1 hexamer [25] and also binds to the human recombinant α5(IV) NC1 domain [27]. Although these studies implicate α3(IV) and α5(IV) chain as the target for X-linked Alport alloantibodies, the nature of mutation(s) leading to a particular genetic defect in these patients was not available. Since our previous study with alloantibodies from an X-linked Alport patient with COL4A5 gene deletion revealed anti-α3(IV) antibodies [15], we addressed the specificity of Alport alloantibodies from an autosomal recessive Alport patient with COL4A3 gene deletion.
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References

PROTEIN ELISA, cZr-rB-h-3-IcMMbS, rB-h-lGEBBbS, rB-h-lGEBBbS.
FUNCTIONAL B-PLACE, sPOTS, sPOTS, sPOTS, sPOTS.
NCT domain in an autoimmune disease, C-PLACE, C-PLACE, C-PLACE.
However, substantial for the first time the presence of 55% (LA) of the
proteins in an autoimmune disease, C-PLACE, C-PLACE, C-PLACE.
Appendix A: Abbreviations

GBM, glomerular basement membrane; TBW, tubular basement mem-

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Acknowledgments

diseases mediated by anti-type I collagen antibodies.

include Alport post-transplant nephritis in the growing list of

for is towards type I collagen. Therefore, it is appropriate to

comparability in the pathogenesis of these diseases [29]. The

The presence of anti-c(Al) collagen antibodies in Goodpasture syn-

However, anti-c(Al) antibodies cannot be completely ruled out.

Lines all antibodies to the c (Al) chain of type I collagen.

The present study identifies the principal target for the cir-

Some weak binding to the c (Al) NCI domain is also observed.

Figure 3. Identification of recombinant human NCI domains that bind

Kalluri et al. Clone Mutation in Alport Syndrome.
and α3(IV) collagen genes in autosomal recessive Alport syndrome. Nature Genet (in press)


