The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/20766

Please be advised that this information was generated on 2019-05-05 and may be subject to change.
A COL4A3 gene mutation and post-transplant anti-α3(IV) collagen alloantibodies in Alport syndrome

RAPID COMMUNICATION

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 COL4A5 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 COL4A5 gene mutation which predicted a loss of 85% of the α3(IV) NCI domain. The specificity of these new antibodies were studied using glomerular basement membrane constituents and recombinant type IV collagen domains. The results establish the target for the alloantibodies from an autosomal recessive Alport patient with COL4A3 deletion as principally the α5(IV) 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 α5(IV) chain [11]. Recently, post-transplant anti-GBM alloantibodies harvested from an 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-α5(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 NC1 domains of the α-chains of type IV collagen. The GBM constituents used were: fibronectin, laminin, heparan sulfate proteoglycan, E, entactin, 7 S, 7 S domain of type IV collagen, peptic solubilized TH, triple helical fragments of type IV collagen, and dimers and monomers of NC1 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.

**Specificity of alloantibodies to bovine type IV collagen NC1 domains by one- and two-dimensional gel electrophoresis and immunoblotting**

The alloantibodies from the affected patient were analyzed by one- and two-dimensional gel electrophoresis and immunoblotting with the bovine NC1 hexamer, bovine α1 + α2 NC1 dimers (D1-dimers) and α3, α4, α5 and α6 NC1 dimers (D2-dimers). At the NC1 hexamer level, the alloantibodies bound strongly to the 54 kDa dimers, a 28 kDa monomer, and very faintly to a 26 kDa monomer (data not shown), as visualized by one dimensional immunoblotting. To further evaluate this binding in terms of type IV collagen α-chains, we performed two-dimensional gel electrophoresis and immunoblotting using bovine NC1 hexamer. The bovine NC1 hexamer was used since all the spots originating from the six α-chains have been identified based on their pl and molecular weight by two-dimensional gel electrophoresis (Identification of α1-α5 spots was previously reported by Gunwar et al [16]). The monomeric and dimeric spots originating from the α6(IV) was recently identified. R. Kalluri, J. Zhou, and B.G. Hudson, unpublished data.) The alloantibodies reacted specifically to the α3(IV) NC1 dimers and monomers, identical to the spots that bind to anti-α3(IV) chain specific antibodies as previously described [16] (Fig. 3). No reactivity was found with the α1(IV), α2(IV), α4(IV), α5(IV) and α6(IV) NC1 dimers and monomers. Additionally, the alloantibodies did not react to the α1 + α2(IV) NC1 dimers and monomers, as evaluated by one dimensional immunoblotting (data not shown). These results establish the target for the alloantibodies as the α3(IV) chain of bovine type IV collagen.

**Inhibition ELISA**

Type IV collagen α-chain specific antibodies were used to perform inhibition ELISA using bovine NC1 hexamer as the antigen. The NC1 hexamer was allowed to bind with one of the six α-chain specific antibodies and subsequently followed with the Alport alloantibodies. The anti-α3(IV) chain specific antibodies inhibited the alloantibodies binding (Figs. 4 A and B). All the other antibodies did not inhibit the alloantibodies binding to the NC1 hexamer. These results establish the alloantibodies as anti-α3(IV)NC1 antibodies.

**Specificity of the alloantibodies to the recombinant human type IV collagen NC1 domains**

The alloantibodies were further analyzed for their capacity to bind recombinant human type IV collagen NC1 domains (α1 to α5). The alloantibodies reacted very strongly to the recombinant α3(IV) NC1 (Fig. 5) and to a very minor degree with the recombinant α5(IV)NC1 domain (1:200 dilution of alloantibera, lost at 1:500). The weak reactivity with the α5(IV)NC1 may be a cross-reactivity phenomenon, due to the high homology between the NC1 domains [24]. Alternatively, the weak binding may be specific reactivity only observed with human α5(IV)NC1 sequence; repeating the gel with half the amount of target NC1 domains eliminated evidence of weak binding while preserving the strong reactivity to α3. These results establish the principal target for post-transplant anti-GBM nephritis in an Alport patient with COL4A3 gene deletion as the α3 chain of type IV collagen.

**Discussion**

In several previous studies, the target for X-linked Alport alloantibodies was identified as the NC1 domain of type IV collagen [10-14]. Hudson et al have shown the target for three post-transplant alloantibodies as the α3(IV) chain [11]. Kleppel et al implicated the α3(IV) chain as a target for alloantibodies in their patients [25, 26]. Their assumption of α5 chain reactivity was
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.
REFERENCES

[16] X-linked hypophosphatemia.
[17] X-linked hypophosphatemia.
[18] X-linked hypophosphatemia.
[22] X-linked hypophosphatemia.
[23] X-linked hypophosphatemia.
[26] X-linked hypophosphatemia.
[27] X-linked hypophosphatemia.

NOTES
Appendix: Abbreviations

Philadelphia, Pennsylvania 19104-6144, USA

CBM, familial basement membrane disease

Regional Meeting in the C. N. A. M. Department of Medicine 700

The present study identifies the principal target for the circuit

The technical assistance of Pamelaloud is gratefully appreciated.

DK-1831, 30200-1919 and Dutch Kidney Foundation Grant 60-722

This study was supported by National Institutes of Health Grants

Acknowledgements

...diseases mediated by anti-type II collagen antibodies. These diseases include: autoimmune thyroiditis, nephritis, and the growing list of other type II collagen-related diseases. Furthermore, it is apparent that this high specificity of 

not-transferable antibody responses can be completely ruled out. However, anti-type II collagen antibodies can be identified in the cytoplasm of 1fA and type 1 collagen. A light alabmogenous stain of type I collagen

The presence of anti-type II (1/3) antibodies in Goodpasture's syndrome

The presence of anti-type II (1/3) antibodies in Goodpasture's syndrome is not surprising, as these antibodies have been observed in post-natal plasmatype II collagen. This finding explains why the (1/3) N. C. I. domain, (1/3) N. C. I. domain, and (1/3) N. C. I. domain in association with a high responder polyrothone, a non-recognition of type II (1/3) antibodies. It is apparent that these antibodies could explain the depressed ability of syngeneic recipient mouse kidney transplants. Therefore, the results from this study could be relevant to the immune system.

Kallunki et al: Clone Mutation in Ahpom's Syndrome
and α3(IV) collagen genes in autosomal recessive Alport syndrome. Nature Genet. (in press)


