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Glomerular Basement Membrane Type IV Collagen in Health and Disease

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Summary. **Glomerular basement membrane is the major supporting structural element of the glomerular capillary wall. This is a highly complex locus which functionally serves as a filtration barrier, and has been the subject of detailed investigation. The composition of whole glomerular basement membrane suggests that collagen is a major component. Isolation and characterization of the collagenous domains has revealed that glomerular basement membrane is chiefly composed of type IV collagen. This molecule has unique properties relating to composition, apparent molecular weight, and the presence of noncollagenous (NC) domains. Following enzymatic digestion of isolated glomerular basement membrane with collagenase, the NC1 domain is released, which represents the C-terminal NC peptides of the type IV collagen molecule. These peptide monomers range in molecular weight (24-28 kD) and have corresponding dimers formed by disulfide bands between adjacent type IV collagen molecules. Two of these NC1 monomer peptides (M26 and M24) are 26kD and 24kD in size, and are identical to NC1 domains of the alpha 1(IV) and alpha 2(IV) collagen chains first characterized in the extracellular matrix of EHS mouse tumor. These are referred to as the "classical type IV collagen chains." Glomerular basement membrane NC1 domains also have two additional newly described NC1 peptides of 28kD ($M28^{+++}$ and $M28^{+}$) which have unique composition, net charge, and antigenicity; the latter are distinct from the classical type IV collagen chains described above and have been designated "novel type IV collagen chains" (alpha 3(IV) and alpha 4(IV) chains). Specific antibody probes have been developed to the M26, M24, $M28^{+++}$ and $M28^{+}$ peptides, which have elucidated the unique distribution of classical and novel type IV collagen chains in mature and developing kidney. Specific assembly processes of classical and novel type IV collagen chains with each other and other basement membrane components are as yet unknown.**

It has now been established that Goodpasture syndrome is an autoimmune disorder mediated by autoantibodies to the NC1 domain of type IV collagen. These autoantibodies react with kidney basement membranes by indirect immunofluorescence in a distribution typical of the novel type IV collagen chains. The Goodpasture epitope has been localized to the alpha 3(IV) NC1 domain.

It is now known that abnormalities of novel type IV collagen chains exist in the glomerular basement membranes in Alport's syndrome (familial nephritis). The known predilection of this disorder in male patients has suggested a genetic defect on the X chromosome. Since the classical type IV collagen chains have been mapped to chromosome 13, this further suggests that the novel type IV collagen chains are distinct entities. The discovery of a third novel type IV collagen chain (alpha 5(IV) chain) based on cDNA sequence analysis, is believed to be the defective element in Alport's syndrome.

CLASSICAL TYPE IV COLLAGEN

Type IV collagen is the major structural component of basement membrane[1,2] and differs from interstitial collagen since type IV collagen is laid down in basement membranes as a procollagen molecule. Type IV collagen has been shown to exist in basement membranes as a triple helix of alpha 1(IV) and alpha 2(IV) chains,[3-8] which are in a ratio 2:1. Classical type IV collagen chains have numerous interruptions in the repeating amino acid triplet sequences of GLY-X-Y, and this likely accounts for the increased flexibility of the molecule and its specialization in basement membranes.[9] The major noncollagenous propeptide portion is located at the carboxy terminal end (NC1) of type IV collagen[5,10] and is responsible for the

drumstick appearance by rotary shadowing electron microscopy; consequently the NC1 region is called the globular domain.[1,11] This domain is therefore composed of the carboxy terminal noncollagenous sequences of both alpha 1(IV) and alpha 2(IV) classical type IV collagen chains which are approximately 26 KD and 24KD respectively, and referred to as M26 and M24 NC1 monomers.[10,12,13] Of the total 330nm length of the type IV collagen chains, NC1 monomers comprise 15-20 nm at the carboxy terminal end. Collagenase digestion of type IV collagen removes most of the collagenous sequences leaving the noncollagenous NC1 domain.[11] Using cDNA probes developed for alpha 1(IV) chains of mouse and human type IV collagen,[14-17] the amino acid sequence of the NC1 domain region has been determined. Two homologous regions of 229 amino acid residues extending from the carboxy terminal of the alpha 1(IV) chains have been defined which have 12 residues of cysteine. Similarly, the complete sequence of the alpha 2(IV) collagen chain has been determined revealing significant differences in composition compared to alpha 1(IV) collagen chain.[18-20] By genomic mapping the genes for alpha 1(IV) and alpha 2(IV) chains have been localized to chromosome 13.[21] Genes for both these type IV collagen chains are oriented in a head-to-head fashion with a common bidirectional promoter which controls gene transcriptional activity.[22,23]

Assembly of type IV collagen molecules into a network is formed in part by interaction of NC1 globular domains at the carboxy terminal ends; the nature of the bonds is not completely known at this time, however, covalent linkages by disulfide bonding, hydrophobic and charge interactions are involved.[10,24] Digestion with collagenase releases the combined globular domains of two type IV collagen molecules consisting of 6 noncollagenous alpha chain monomers, and hence the proposed composition of NC1 hexamers having 160 KD size.[12] Since the NC1 hexamers are held together by hydrophobic and charge interactions, SDS PAGE under non-reducing conditions reveals bands at 24-26 KD (monomers) and 42-52 KD (dimers) using NC1 domain from type IV collagen of the EHS mouse tumor.[10] Studies from our laboratory[13,15] and Butkowski[26,27] revealed a greater complexity of NC1 monomers in human and bovine glomerular basement membrane (GBM).

Further assembly of classical alpha (IV) collagen into a network has been observed by disulfide bonding of four molecules at the amino terminal into the 7S domain; lateral associations and binding of these molecules has been proposed.[11,28,29]

NOVEL TYPE IV COLLAGEN CHAINS

When we examined NC1 hexamers by SDS PAGE from human GBM collagenase digests we observed multiple bands in the dimer and monomer regions.[25] This complexity was more apparent by 2-dimension gels using nonequilibrium pH gradient electrophoresis (NEPHGE) in the first dimension followed by SDS PAGE, which permitted definition of human GBM specific components in the dimer (D54) and monomer (M28) regions. By comparing EHS mouse tumor NC1 D52/43 dimers and M26/24 monomers, we observed that these classical type IV collagen NC1 domain components were cationic pH 8.0 ($D52^{++}$, $D43^{++}$, $M26^{++}$) and neutral pH 7.0 (D43, D40, M24).[30] Although the latter were also found in human GBM NC1 domain, the human GBM D54 dimers and M28 monomers had both a very cationic charge pH >9.5 ($D54^{+++}$, $M28^{+++}$) as well as a mildly cationic charge pH 7-8 ($D54^{+}$, $M28^{+}$) (Fig. 1). It therefore became apparent that NC1 domain from EHS tumor matrix differed from human GBM NC1 by absence of the human GBM components $D54^{+++}$ and $D54^{+}$ dimers, and $M28^{+++}$ and $M28^{+}$ monomers. These components are derived from the NC1 domains of novel alpha 3(IV) and 4(IV) collagen chains.[3] When the individual NC1 components in the EHS tumor and human GBM were separated by HPLC reverse phase chromatography (C18 Vydac) using acetonitrile gradients, it was observed that NC1 D52/43 monomers eluted at 31-33% acetonitrile. The human GBM cationic dimers and monomers eluted at 34-36% acetonitrile.[26,30]

Butkowski, Wieslander, and Hudson have characterized the NC1 domain of type IV collagen in collagenase digests of bovine GBM.[12,26] Components of the domain include monomer and dimer-size polypeptides. Reverse phase HPLC analysis of the globular domain of bovine GBM revealed a more heterogeneous composition than would be predicted if this domain were identical to the NC1 domain from EHS tumor. Four monomeric subunits (M1, M1a, M2*, and M3) were found, whereas NC1 from the EHS tumor contained only two monomeric subunits. Monomer M2* was uniquely reactive with Goodpasture sera discussed below. Amino terminal sequence analysis has demonstrated that M1 and M1a corresponded to the non collagenous regions of the alpha 1(IV) and alpha 2(IV) classical collagen chains respectively.

The amino terminal sequences of M2* and M3 are

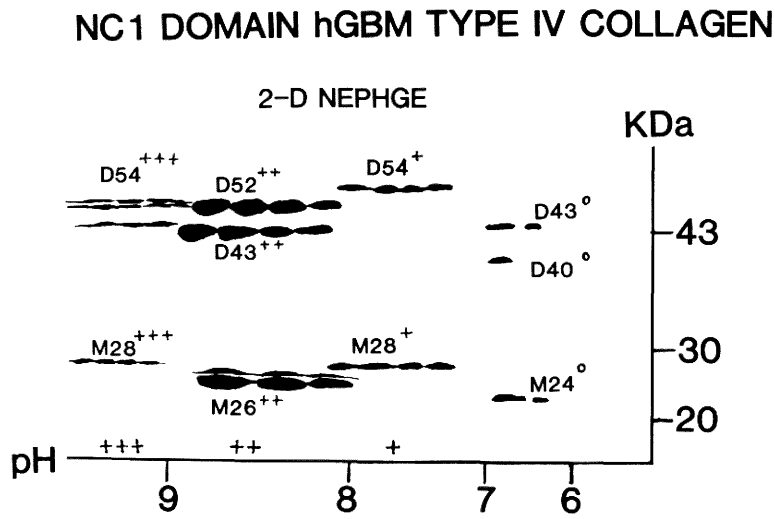

Fig. 1 Commassie stain of 2-dimension NEPHGE/SDS PAGE of human GBM NC1 domain (schematic drawing) showing various cationic charged D54-D40 dimers and M28-M24 monomers. Note: Four human GBM NC1 monomers $M28^{+++}$, $M28^{+}$, $M26^{++}$, and M24 are evident.

distinct from M1 and M1a. These monomers were isolated from various bovine tissues including GBM (12) and anterior lens capsule basement membrane.[27] That M2* and M3 are derived from collagen chains is suggested by the occurrence of GLY-X-Y triplets in their sequence. M2* and M3 each have monomer and dimer forms, and each demonstrates similar behavior in denaturing solvents. M2* and M3 are derived from alpha 3(IV) and alpha 4(IV) collagen chains.[31]

Studies from our laboratory have dealt with isolation and characterization of the NC1 domain components of human GBM. In view of the cationic charge properties of the human NC1 domain subunits, it was observed that ion exchange chromatography on S-Sepharose permitted separation of the unbound 7S domain and M26 and M24 classical chain components; the M28 NC1 novel collagen chain subunits were eluted from the column uning a NaCl gradient.[32,33] $M28^{+++}$ and $M28^{+}$ were further purified from the M28 containing pool of the S-Sepharose cation exchange column. This pool is highly enriched in M28 antigens, which can be resolved from each other and from contaminating M24/26 by reverse phase HPLC in an acetonitrile gradient. The purity of the isolated human GBM monomers was established by their homogeneity by SDS PAGE.

Amino-terminal sequences of the isolated human monomers (Table 1), along with Western blotting results described below, allowed us to identify M26 and M24 as being derived from classical type IV collagen alpha 1 and 2 chains, respectively. The sequence obtained for $M28^{+++}$ indicates that it corresponds to bovine M2* (alpha 3(IV) NC1), and the sequence of $M28^{+}$ corresponds to bovine M3 (alpha 4(IV) NC1).

Table 1.

Amino-terminal sequences of human GBM collagen globular domains

Monomer	Amino-terminal sequence
M26	GPP+GTP+SVDXXFLV
M24	GMP+GRSVSIGYLLVK
$M28^{+++}$	GLLGKP+GDTGPP+A
$M28^{+}$	GPP+GFGPPGYLGPFLLVL

P+ indicates hydroxyproline. Each monomer begins with one or more GLY-X-Y triplets, indicative of a collagenous origin. Underlined portion of each subunit corresponds to the non collagenous (NC1) regions.[32]

TISSUE LOCALIZATION OF NOVEL TYPE IV COLLAGEN CHAINS

We have recently extended our previous observations that there is regional variation in the distribution of type IV collagen chains in the glomerular capillary wall and blood vessels.[34,35] Using specific antibody probes which recognize M24, M26, and $M28^{+}$ monomers, we have documented that alpha 1(IV) and alpha 2(IV) chains are present in the glomerular capillary subendothelial space, and also readily detected in the

glomerular mesangium, Bowman's capsule, tubular basement membrane, and blood vessel walls.[35,36] In contrast, novel type IV collagen chains containing $M28^{+}$ and $M28^{+++}$ monomers are only found in the central region of the GBM, focally in some tubular basement membranes, and are not detectable in blood vessel walls (Fig. 2). These findings establish the important distinction that there is regional variation in assembly of type IV collagen chains in renal basement membranes which is reflected in the unique distribution of novel type IV collagen chains to the central region of the GBM.[34–36]

Further evidence suggesting that classical and novel type IV collagen chains have distinct and separate origins, can be found in their unique distribution in the basement membranes of developing kidney during embryogenesis.[34–37] The reactivity of antibodies to alpha 1(IV) and alpha 2(IV) chains was observed at all stages of nephron development with staining of all basement membranes of tubules, blood vessels, vesicle cleft, the S-form and early/late capillary loop stages of glomerular development. In contrast, detection of M28 NC1 subunits of novel collagen chains was observed only late in nephron development corresponding to the change of the glomerular S-form to the early capillary stage. Additionally, these M28 peptide subunits are found only in distal tubular basement membranes, Bowman's capsule, and GBM of the developing nephron units (Fig. 3).

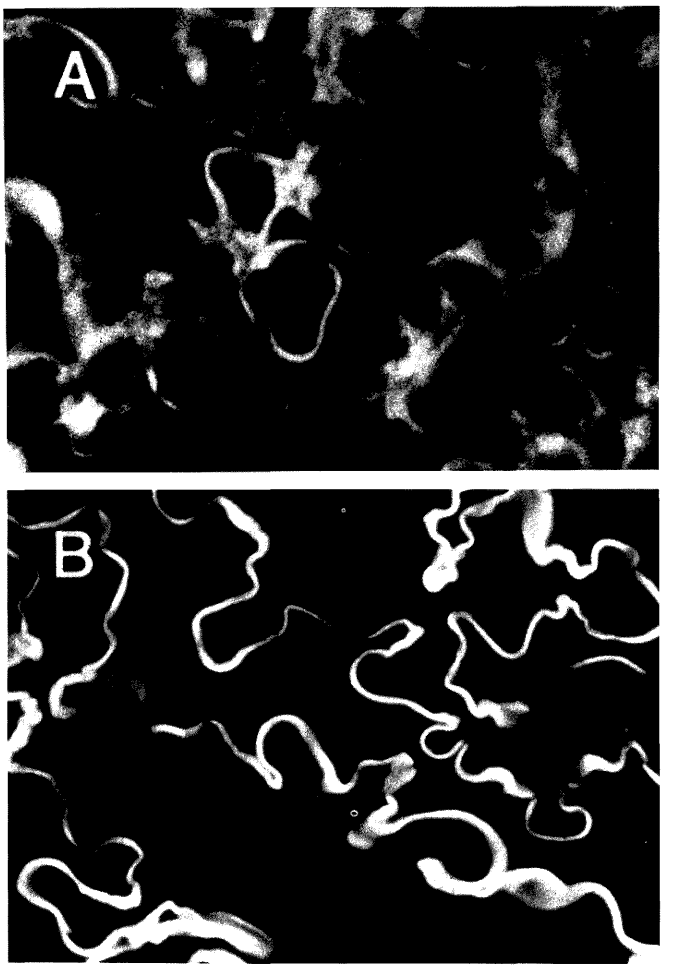

Fig. 2 Immunofluorescent staining of the glomerular capillary bed. A. Mesangial matrix and subendothelial staining of classical collagen chains (NC1 alpha 1(IV) domain). B. Central GBM staining distribution of novel collagen chain (NC1 alpha 4(IV) domain). Magnification ×1200 (with permission from reference 36).

Fig. 3 Immunofluorescent staining of human fetal kidney cortex showing progressive maturation of glomeruli; reactivity with GBM and distal tubular basement membrane for alpha 3(IV) NC1 domain is shown. Magnification ×250 (with permission from reference 37).

GOODPASTURE SYNDROME

Some individuals with glomerulonephritis and lung hemorrhage have linear deposition of IgG along the GBM identical to experimental models of nephritis in animals using heterologous anti-GBM antibodies.[38] Circulating antibodies to GBM are detectable in some of these patients by indirect immunofluorescence on normal human kidneys sections.[39,40] Antibody bound to renal basement membranes in Goodpasture syndrome has been eluted from nephrectomy specimens using low pH buffers, neutralized and shown to fix to GBM of normal human kidney sections.[40] More sensitive techniques to measure circulating GBM autoantibodies by ELISA assay have been developed for diagnosis and monitoring treatment.[41,42] The development of anti-GBM assays depended upon establishment of methods to solubilize human GBM while preserving antigenicity of Goodpasture determinants: collagenase or trypsin digestion, 4M urea and 6M guanidine[43] extraction were effective. Pepsin digestion extraction with SDS and mercaptoethanol resulted in total loss of antigenicity.

Wieslander and co-workers[12,44] initially purified human GBM collagenase digests by using material which did not bind to DE52 anion exchange columns in urea, and was then applied to CM cellulose cation exchange columns; the ELISA reactive material was eluted with 0.05-1M NaCl gradient. By Western blotting with Goodpasture antibodies reactive material in the 50-54 KD and 24-28 KD regions was revealed. Gel filtration under denaturing conditions in 6M guanidine suggested that the 50-54 KD and 24-28 components were dimers and monomers respectively.[12,44] Studies from our laboratory using NC1 hexamers obtained by gel filtration under non-denaturing conditions revealed similar findings by Western blotting to detect Goodpasture reactive material.[45]

To extend our observations of multiple dimers and monomers of varying charge in human GBM NC1 domain, we examined charge heterogeneity of the Goodpasture reactive antigen(s). Because of the cationic nature of most components, improved resolution was achieved using NEPHGE in the first dimension. When the components were resolved in the second dimension using 8-18% SDS PAGE gels. It was observed by immunoblotting of 2-dimension gels using Goodpasture anti-GBM autoantibodies that $D52^{++}$, $D54^{+}$, and $M26^{++}$, $M28^{+}$ NC1 subunits were reactive, however, the greatest reactivity was seen

Table 2. Comparison of human GBM NC1 domain subundts with bovine GBM and EHS tumor subunits

KD	HUMAN GBM		BOVINE GBM		EHS TUMOR	
Monomers	NC1 Subunit	GP Reactivity	NC1 Subunit	GP Reactivity	NC1 Subunit	GP Reactivity
28	$M28^{+++}$	4+	M2*	1+	Absent	N/A
	$M28^{+}$	1+	M3	NEG	Absent	N/A
26	$M26^{++}$	1+	M1	NEG	$M26^{++}$	TR
24	M24	TR	M1a	NEG	M24	TR

NEG = Negative; N/A = Not Applicable; GP = Goodpasture antibody

with $D54^{+++}$ and $M28^{+++}$ very cationic components, which are derived from the alpha 3(IV) collagen chain (Table 2). Goodpasture sera, therefore, reacted with most dimers and monomers except the neutral D43 and M24 and weakly cationic $M28^{+}$. The corresponding D43 and M24 neutral subunits of EHS mouse tumor NC1 domain are derived from alpha 2(IV) classical collagen chains, and the $D52^{++}$ and $M26^{++}$ are from alpha 1(IV) chains; thus although Goodpasture antibodies appear to react with subunits from alpha 1 type IV chains, as well as with several new subunits not present in the EHS mouse tumor NC1 domain.[25]

We further analyzed Goodpasture antibody reactive components in human GBM.[30] $M28^{+++}$ and $M28^{+}$ monomers of human GBM could be enriched from other NC1 components by gel filtration. 2) $M28^{+++}$ monomer was the most reactive with Goodpasture antibody and was present only in human GBM. 3) $M28^{+++}$ and $M28^{+}$ components have a high half cystine content and homology with the Goodpasture reactive $M2^{+}$ components in bovine GBM. 4) By ELISA inhibition assay, purified human GBM M28 monomers are 30-fold more active than M24/26 monomers in binding activity with Goodpasture antibody. It appears that Goodpasture antibody reacts primarily with $M28^{+++}$ (human)[30] and M2* (bovine) monomers.[46-48] However, there is also reactivity with $M26^{++}$ components and related dimers in the human material only (Table 2). Recent observations from this laboratory suggest that the other NC1 domain subunits contain small amounts of cross reactive Goodpasture epitope(s) (H. Matsukura, R. Butkowski, and A. J. Fish, manuscript in preparation). Goodpasture antigen has also been identified in lung and placental basement membranes.[32,49,50-52]

ALPORT'S FAMILIAL NEPHRITIS

Alport's syndrome is an X-linked genetic disorder characterized by progressive glomerular basement membrane disruption in affected males and a milder, less severe, carrier state in females. In this disorder there is associated high frequency sensorineural hearing loss and ocular lens abnormalities.[53,54]

The GBM abnormalities in Alport's syndrome include early splitting of the lamina densa and ultimate progressive alteration of the GBM leading to a basket-weave laminated appearance.[55,56] The earliest suggestion of a biochemical defect in Alport GBM was the observation that Goodpasture sera which fixed to the GBM of normal human kidney GBM by indirect immunofluorescence did not fix to the GBM in kidney sections of Alport kidneys.[57-61] Since the Goodpasture epitope is present on the NC1 domain of alpha 3(IV) chains, this provided the first evidence for involvement of novel collagen chains. It is known that heterogeneity of basement membranes exists and it was subsequently shown that certain extra renal basement membranes, which contained novel collagen chains, were also abnormal in Alport syndrome.[62] In addition, it was observed that most involved basement membranes also lack the alpha 4(IV) novel collagen chain as well.[63,64]

Studies from our laboratory in this area were facilitated by an autoantibody to GBM from an Alport syndrome patient which developed following renal transplantation. This antibody (FNS1) reacted with GBM and epidermal basement membranes of normal skin by indirect immunofluorescence, however, was non-reactive with Alport tissues.[63] By Western blotting, FNS1 did not react with $M28^{+++}$ or $M28^{+}$ NC1 subunits of type IV novel chain collagen, but reacted with a 26KD NC1 component and its corresponding dimers.[25,64] FNS1 did not react with

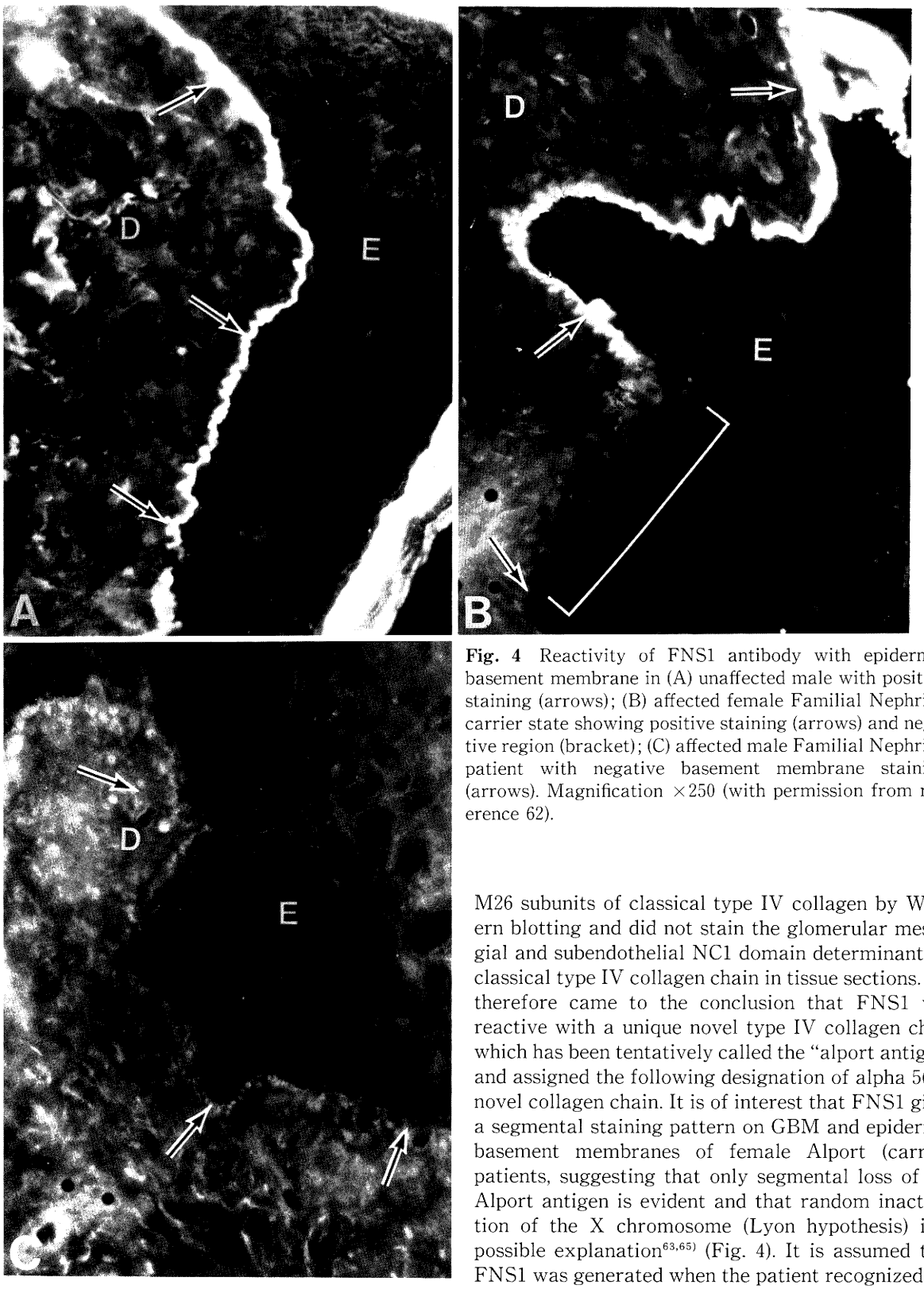

Fig. 4 Reactivity of FNS1 antibody with epidermal basement membrane in (A) unaffected male with positive staining (arrows); (B) affected female Familial Nephritis carrier state showing positive staining (arrows) and negative region (bracket); (C) affected male Familial Nephritis patient with negative basement membrane staining (arrows). Magnification ×250 (with permission from reference 62).

M26 subunits of classical type IV collagen by Western blotting and did not stain the glomerular mesangial and subendothelial NC1 domain determinants of classical type IV collagen chain in tissue sections. We therefore came to the conclusion that FNS1 was reactive with a unique novel type IV collagen chain which has been tentatively called the "alport antigen" and assigned the following designation of alpha 5(IV) novel collagen chain. It is of interest that FNS1 gives a segmental staining pattern on GBM and epidermal basement membranes of female Alport (carrier) patients, suggesting that only segmental loss of the Alport antigen is evident and that random inactivation of the X chromosome (Lyon hypothesis) is a possible explanation[63,65] (Fig. 4). It is assumed that FNS1 was generated when the patient recognized the Alport alpha 5(IV) chains in the transplanted kidney

and an autoimmune response ensued. The Alport locus has been mapped to the long arm of the X chromosome within the Xq 21.2-22.1 region[66,67] based on restriction fragment length polymorphisms.

It therefore has been our hypothesis that FNS1 autoantibody is a marker for the NC1 domain of the novel basement membrane alpha 5(IV) collagen chain and that a genetic mutation involving synthesis and GBM incorporation of this chain is the basis of the Alport syndrome defect. It appears that the NC1 subunit of alpha 5(IV) has an apparent molecular weight of 26KD with a similar cationic charge by 2 dimension NEHPGE SDS PAGE analysis compared to the M26 NC1 subunit of the alpha 1(IV) novel collagen chain. Differences in tissue localization in normal and Alport renal and extrarenal basement membranes suggest that these 26KD NC1 domain subunits are distinct and of different collagen chain origin.[54,65]

More recent studies from the University of Oulu, Finland, have confirmed the presence of a third novel type IV collagen chain, the composition of which has been determined from the nucleotide sequence of cDNA clones encoding this protein. In view of the distinct amino acid sequence of the NC1 domain, which has homology with alpha 3(IV) and alpha 4(IV) NC1 (Table 3), this region has been designated alpha 5(IV) NC1.[68] Since this gene (COL4A5) has been identified on the long arm of the X chromosome, it is believed to be the Alport locus. Another report of alpha 5(IV) chain has been described by Myers and co-workers.[69] It remains to be confirmed that FNS1 autoantibody is reacting with the NC1 domain of the chain encoded by the COL4A5 gene. Other autoantibodies reported in Alport patients after renal transplantation have broader reactivities with other NC1 domain subunits.[70,71] Further studies in two Alport families have revealed two different point mutations based on analysis of genomic DNA[72] It therefore appears that final characterization of the Alport gene is imminent and that different mutations of this gene may result in expression of the Alport syndrome.

ASSEMBLY OF GLOMERULAR BASEMENT MEMBRANE COMPONENTS

The discussion above has dealt in detail with the current state of knowledge regarding the collagenous components of GBM. While considerable information regarding the biochemical composition of the various type IV collagen chains is available, the intermolecular assembly of these triple chain helices is unknown. Observations of the assembly of classical type IV collagen chains from the EHS mouse tumor have been made by electron microscopy of rotary shadowed replicas.[9,11,29] In these studies of the mouse tumor matrix, it was observed that a network of classical chain collagen molecules was formed through the carboxy terminal NC1 globular domains and the amino terminal 7S domains, along with lateral associations between these joined molecules to form a polygonal network.

With respect to the novel type IV collagen chains, little is known about their assembly into networks. The novel chain NC1 domains have disulfide bonded dimers[10,26] and therefore these molecules are joined at their carboxy terminals but it is not known whether collagenous 7S regions of the novel chain N terminals exist. Similarly, it is not known whether heterodimers between classical and novel collagen chains exist, or whether heterodimers of NC1 domain subunits can form although they have been postulated.[47,48] In general, it appears that the novel

Table 3. Homology of amino-terminal sequence analyses of NC1 subunits of novel and classical type IV collagen chains*

Alpha 3(IV)	GLXGKPGDTGPP|AAGAVMRGFVFTRHSQTTAI
Alpha 4(IV)	GPPGF|GPG YLSGFLLVLHSQTDGEPT PMG
Alpha 5(IV)	GPDGLQGPPGPPGTS|SVAHGFLITRHSQTTDAPQCPQGT
Alpha 1(IV)	GPDGLPGSMGPPGTP|SVDHGFLVTRHSQTIDDZVCPPG
Alpha 2(IV)	GRPGSPGLGRS|VSIGYLLVKHSQTDQEPMCPVG

* Sequence homologies range from 38-53% (modified from references 31, 68, 69). Gaps in the alpha 2 (IV) and alpha 4(IV) sequences have introduced for maximal allignment. The vertical line represents the beginning of the non-collagenous (NC1) domain. A common consensus sequence of hsqt in the noncollagenous region is shown in the boxed area.

chains comprise a lower percentage of total GBM type IV collagen, but this may reflect more extensive cross-liking of these chains in the central lamina densa portion of the GBM and hence, a greater resistance to solubilization with collagenase.

It is beyond the scope of this article to cover in detail the several additional glycoproteins which are known to present in the GBM. These components are likely to play as yet undefined roles in structure and function of the GBM. Laminin is a cross-shaped glycoprotein consisting of B1, B2, and A chain components.[9,73] It is known to interact with a dumbell-shaped molecule called entactin/nidogen[9] which itself has binding sites with type IV collagen. A large heparan sulfate proteoglycan contains anionic charge sites important in forming the filtration barrier of the GBM. Additionally, this molecule has binding sites to interact with laminin and type IV collagen. Finally, important functional aspects of these molecules lies in their defined cell binding sites where interactions with cell membrane integrin receptors[9] occurs. Most of these cell binding functions have been examined in vitro, however, it is likely that the cell binding sites of these basement membrane glycoproteins play an important role in the anchoring and function of endothelial and epithelial cells of the glomerular capillary wall.

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