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An immunohistochemical and electron microscopic study of extra-renal basement membranes in dogs with Samoyed hereditary glomerulopathy *

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Summary. Samoyed hereditary glomerulopathy (SHG) in dogs has been employed as a model for human hereditary nephritis (HN), since affected dogs and patients show splitting of glomerular capillary basement membranes by electron microscopy (EM) and absent staining of glomerular capillaries for Goodpasture antigen (GPA) by immunofluorescence (IF). EM and IF were used to examine basement membranes (BM) in skin, lung, choroid plexus, lens, retina, and inner ear in SHG. By EM, BM in these tissues appeared similar in affected male, carrier female, and unaffected dogs. By IF, GPA could be detected only in lens capsule, internal limiting membrane of retina and basilar membrane of inner ear of unaffected and carrier female dogs, but not in affected male dogs. However, eye abnormalities and hearing loss were not present in any dogs, in contrast to their frequent occurrence in human HN. Our findings on extra-renal BM in SHG suggest that GPA is not required to maintain normal vision or hearing in affected male dogs and permit a greater understanding of the pathogenesis of human HN.

Key words: Hereditary nephritis – Goodpasture antigen – Basement membrane

Introduction

Hereditary nephritis (HN) in man consists of various inherited glomerular diseases, some of which show multilaminar splitting of glomerular capillary basement membranes (GCBM) by electron microscopy (EM) (Grünfeld 1985; Gubler et al. 1981; Habib et al. 1982). Recently, immunofluorescence (IF) studies have demonstrated laminin, collagen type IV, and fibronectin in glomeruli of patients with HN (Habib et al. 1982; Melvin et al. 1986), but an absence of Goodpasture antigen in most affected males and some affected females (Jenis et al. 1981; Jeraj et al. 1983; McCoy et al. 1982; Olson et al. 1980). In contrast to the well documented changes seen by EM and IF in glomeruli of patients with HN, there has been no detailed description of basement membranes (BM) of extra-renal tissues. Such BM may not be normal, since patients with HN often have eye, ear, and other extra-renal abnormalities (Grünfeld 1985; Gubler et al. 1981; Habib et al. 1982).

We have been studying a family of Samoyed dogs with hereditary glomerulopathy (SHG) as a model for human HN because the two diseases have a number of similarities. First, SHG in dogs produces premature death from renal failure in males but not in females and hence, is inherited as an X-linked dominant trait, similar to some forms of human HN (Jansen et al. 1986a, b; Jansen et al. 1987). Second, male dogs with SHG show severe multilaminar splitting of GCBM by EM which is identical to that seen in human males with HN, while carrier female dogs show a milder lesion of GCBM by EM (Jansen et al. 1986a). Third, IF has demonstrated that GPA is absent from glomerular capillary walls of affected male dogs with SHG, as in human males with HN, but is present in carrier female and unaffected dogs (Thorner et al. 1987). However, some differences do exist, since affected dogs do not consistently have hematuria, and do not show marked thinning of GCBM, as has been noted in HN (Grünfeld 1985; Habib et al. 1982).

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In the present study, EM and IF were used to examine BM in various extrarenal tissues (e.g., skin, lung, choroid plexus, lens, retina, and inner ear) of affected males with SHG, carrier females, and unaffected dogs. It was felt that such a study might provide a structural basis for understanding the extra-renal abnormalities often seen clinically in human HN, and yield additional information on the pathogenesis of this disease.

Materials and methods

For performance of morphological studies using light microscopy (LM), EM, and IF, dogs from a pedigree with SHG were categorized as affected males (12 dogs 4.0 to 8.5 months of age), carrier females (4 dogs 4.5 to 60 months), and unaffected (14 dogs 4.0 to 15 months), as previously described (Jansen et al. 1986a). Tissues (skin, lung, choroid plexus, retina, and lens) were taken at euthanasia, fixed in buffered formalin, and processed for LM by standard methods. The inner ear was also removed at euthanasia together with the surrounding bone, fixed in buffered formalin, decalcified, and processed en bloc for LM. Five micron thick tissue sections were prepared and stained with hematoxylin and eosin, and periodic acid silver methenamine.

For examination by EM, tissues were fixed by immersion for at least 1 hour in a mixture of 4% paraformaldehyde-1% glutaraldehyde, postfixed in osmium tetroxide, and embedded in an Epon-Araldite mixture. In the case of the inner ear, the cochlea was perfused with fixative via the round and oval windows and the stria vascularis, basilar membrane, and tectorial membrane were removed separately. Sections were cut at 50 nm, stained with uranyl acetate and lead citrate, and examined at 60 kV on a Philips 300 electron microscope. LM and EM were used to examine epidermal-dermal, adnexal, vascular, and perineural BM in skin; alveolar, vascular, bronchial, and perineural BM in lung; epithelial and vascular BM in choroid plexus; lens capsule; internal limiting membrane in retina; and stria vascularis, basilar membrane, and tectorial membrane in inner ear.

For examination by IF, tissues were snap-frozen in liquid nitrogen, stored at −70° C until use, and then cut at 5 μ. Sections were picked up on gelatinized slides, treated with rabbit antibodies or human serum, and stained using fluoresceinated goat anti-rabbit or rabbit anti-human IgG antiserum (Cappel, Cockranville, PA, USA). In the case of the inner ear, the temporal bone was snap-frozen and stored at −70° C, following which the inner ear was dissected out. In some experiments, tissue sections were treated with acid urea to expose hidden GPA, as previously described (Yoshioka et al. 1985), before they were stained with human serum and fluoresceinated anti-human IgG. Human skin served as a positive control in the acid urea experiments and showed staining of BM after, but not before, acid urea treatment. Normal rabbit and human sera were used as negative controls.

Immune rabbit sera employed for IF contained the following polyclonal antibodies: anti-laminin and anti-fibronectin (Bethesda Research Lab., Gaithersburg, MD, USA), both used at a 1:50 dilution, and anti-collagen type IV, prepared by us by immunizing rabbits with collagen type IV isolated from human placenta by pepsin digestion (Glanville et al. 1979), used at a 1:20 dilution. The specificity of these antibodies was previously demonstrated by radioimmunoassay (Thorner et al. 1987). In addition to the rabbit antibodies, human serum obtained from a patient with Goodpasture syndrome was used, which contained antibody that was reactive with human and cross-reactive with dog GPA (Thorner et al. 1987).

For assessment of vision, 6 affected males, 2 carrier females, and 8 unaffected dogs were observed at 4 months of age for their perception of moving objects. Examination of the lens was performed by slit lamp illumination, while the ocular fundus was observed by ophthalmoscopy.

For subjective assessment of hearing, 3 affected males and 7 unaffected dogs were observed at 6 months of age in a quiet room to determine their response to a dog whistle, which emits frequencies above 20 kHz. In addition, auditory thresholds were objectively assessed in 5 affected males and 7 unaffected dogs using standard brain stem evoked response techniques (Lev and Sohmer 1972).

Results

LM of extra-renal tissues

BM of skin, lung, choroid plexus, lens, retina, and inner ear appeared similar in unaffected, affected male, and carrier female dogs.

EM of extra-renal tissues

EM of BM of skin, lung, choroid plexus, lens, retina, and inner ear failed to demonstrate any differences among unaffected, affected male, and carrier female dogs. Electron micrographs are shown only for affected male dogs (Fig. 1). Of particular interest was the observation that the tectorial membrane consisted of thin filamentous material, arranged in an unorganized fashion (Fig. 1h), and did not resemble a BM morphologically.

IF of extra-renal tissues

Unaffected dogs. Laminin and collagen type IV were detected in all BM of the skin, lung, choroid plexus, lens, retina, and inner ear (Table 1, Fig. 2a–2f). In the case of the inner ear, staining was seen in the stria vascularis (i.e., underlying the surface epithelium and around blood vessels), around the nerves of the spiral ganglion, and along the basilar membrane, in a fine linear pattern which corresponded to the subepithelial BM rather than its entire width (Fig. 2f). There was no staining of the tectorial membrane for laminin or collagen type IV.

Staining for fibronectin was seen in interstitial and perivascular regions of skin, lung, choroid plexus, and stria vascularis of the inner ear. Fibronectin staining was also observed throughout the entire thickness of the basilar membrane of the inner ear, including the subepithelial, fibrillar, and amorphous areas seen by EM. There was no staining for fibronectin in the lens capsule, internal lim-

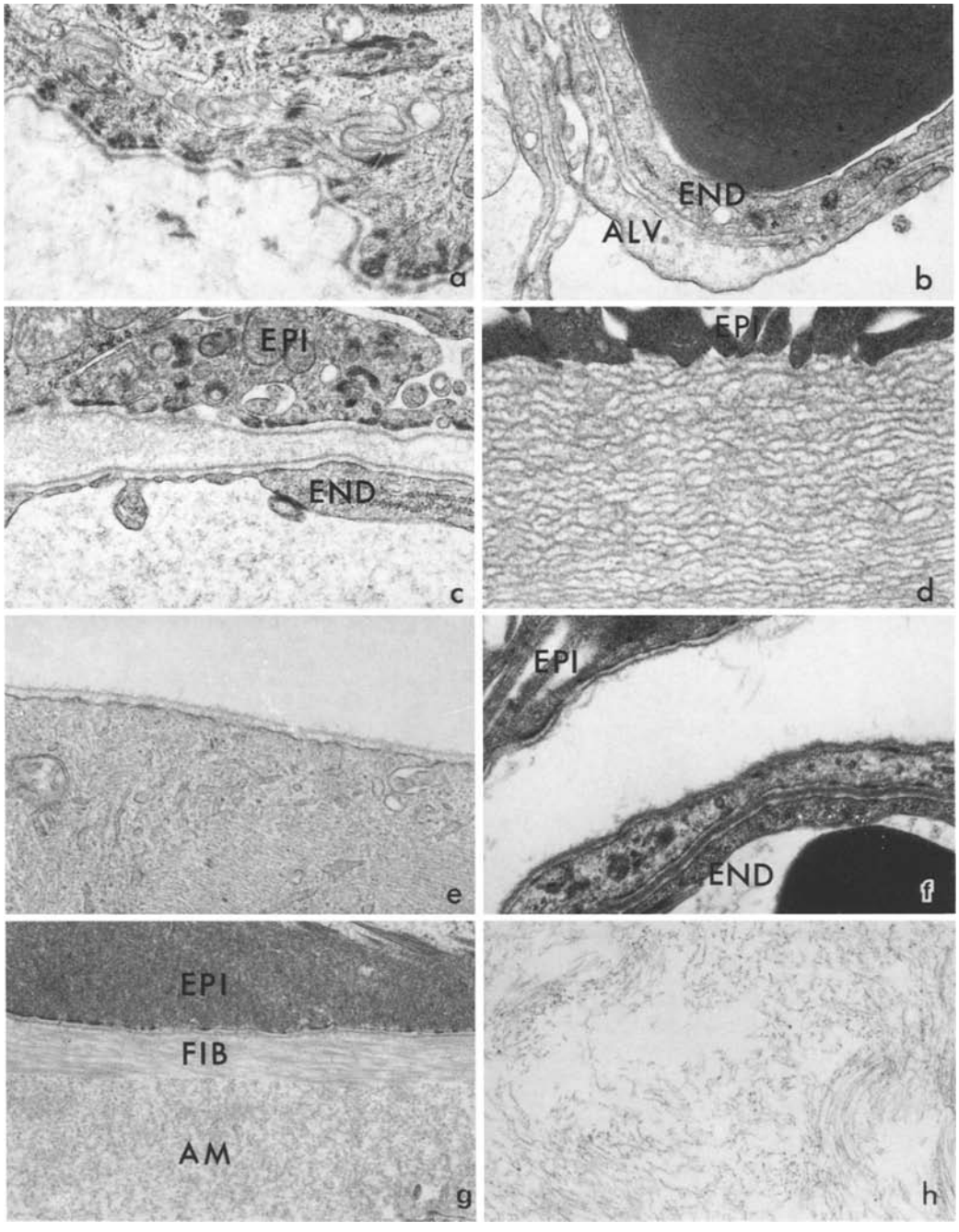

Fig. 1. Electron microscopy of extra-renal basement membranes (*BM*) of an affected male dog. Normal BM are seen in (**a**) skin, at the epidermal-dermal junction (×18400); (**b**) lung, between capillary endothelium (*END*) and alveolar lining cells (*ALV*) (×23000); (**c**) choroid plexus, beneath the choroidal epithelium (*EPI*) and capillary endothelium (*END*) (×18400); (**d**) lens capsule, which shows multiple layers of BM-like material adjacent to the lens epithelium (*EPI*) (×18400); (**e**) internal limiting membrane of the retina (×36000); (**f**) stria vascularis of the inner ear, in which a BM underlies the surface epithelium (*EPI*) and capillary endothelium (*END*) (×18400); (**g**) basilar membrane of the inner ear, in which a BM underlies the epithelium (*EPI*) of the organ of Corti. Beneath this is a fibrillar layer (*FIB*) adjacent to an amorphous material (*AM*) and bordered by another cell layer (×12000); (**h**) tectorial membrane, which consists of an unorganized arrangement of fibrillar material separated by electron-lucent areas and does not resemble a BM morphologically (×18400)

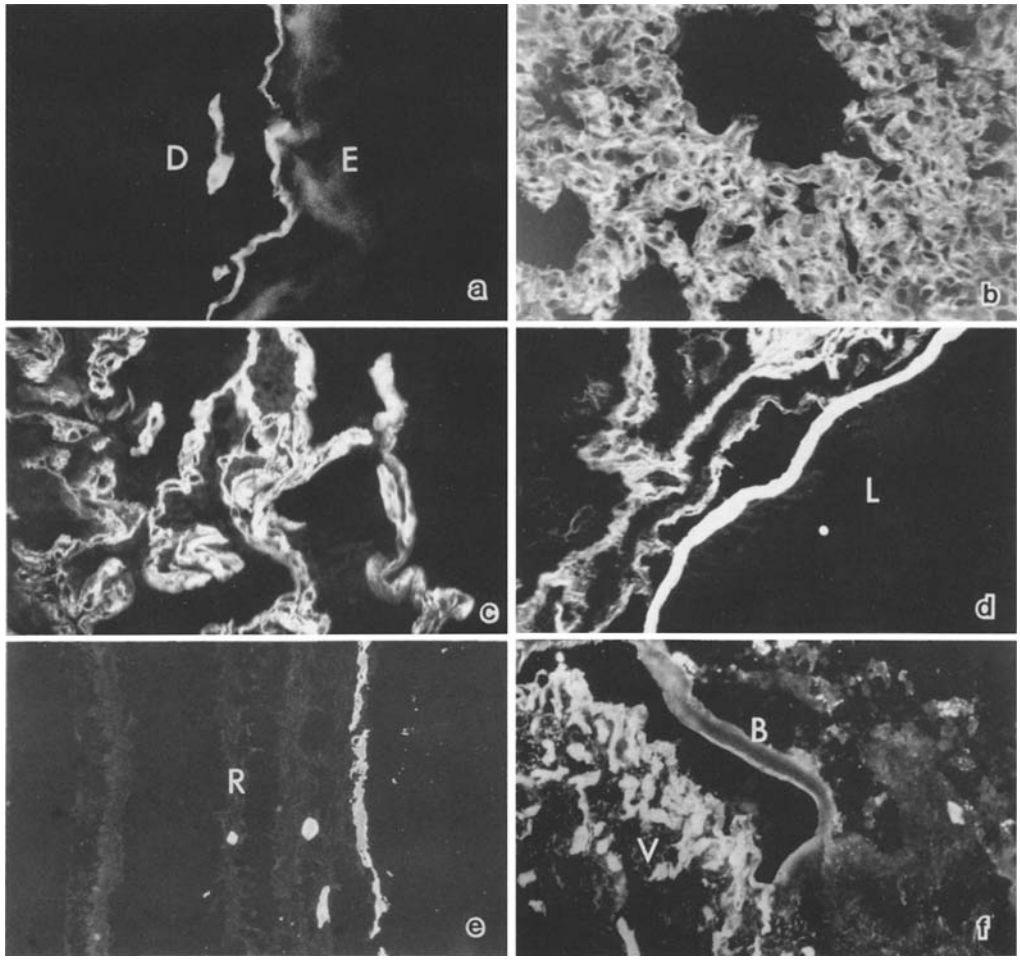

Fig. 2. Immunofluorescent staining of extra-renal basement membranes with anti-laminin antibody (**a–f**) in an unaffected dog, and with Goodpasture serum (**g–k**) in an unaffected and affected male dog. Staining for laminin is seen in (**a**) skin, at the epidermal-dermal junction and around the dermal capillaries (×224); (**b**) lung, along alveolar septa (×224); (**c**) choroid plexus, in subepithelial and perivascular locations (×224); (**d**) lens capsule (×224); (**e**) retina, in the internal limiting membrane (×224), and (**f**) inner ear, in the basilar membrane and the stria vascularis (×224) of an unaffected dog. Staining for Goodpasture antigen (*GPA*) is seen in (**g**) lens capsule (×224), (**h**) internal limiting membrane of the retina (×224), and (**i**) basilar membrane of the inner ear (×224) of an unaffected dog. Staining of retinal blood vessels is nonspecific. There is no staining for GPA in (**j**) lens capsule (×224) and (**k**) internal limiting membrane (×224) of an affected male dog. (*D*=dermis, *E*=epidermis, *L*=lens, *R*=retina, *B*=basilar membrane, *V*=stria vascularis)

iting membrane of the retina, or tectorial membrane of the inner ear.

Staining for GPA was seen in the lens capsule (Fig. 2g), internal limiting membrane of the retina (Fig. 2h), and basilar membrane of the inner ear (Fig. 2i), but not in the skin, lung, choroid plexus, or stria vascularis or tectorial membrane of the inner ear. These tissues remained negative for GPA even after acid-urea treatment of tissue sections.

Affected male dogs. Staining for laminin, collagen type IV, and fibronectin in affected male dogs was similar to that in unaffected dogs. In addition, no staining for GPA was seen in skin, lung, choroid plexus, or stria vascularis or tectorial membrane of the inner ear. However, unlike unaffected dogs, GPA was not detected by IF in the lens capsule (Fig. 2j), internal limiting membrane of the retina (Fig. 2k), or basilar membrane of the inner ear of affected male dogs. Treatment of the lens, retina, and inner ear with acid urea failed to expose GPA (Table 1).

Carrier female dogs. Results in carrier females were identical to those in unaffected dogs (Table 1).

Vision studies

All unaffected, affected male, and carrier female dogs followed moving objects with their eyes and

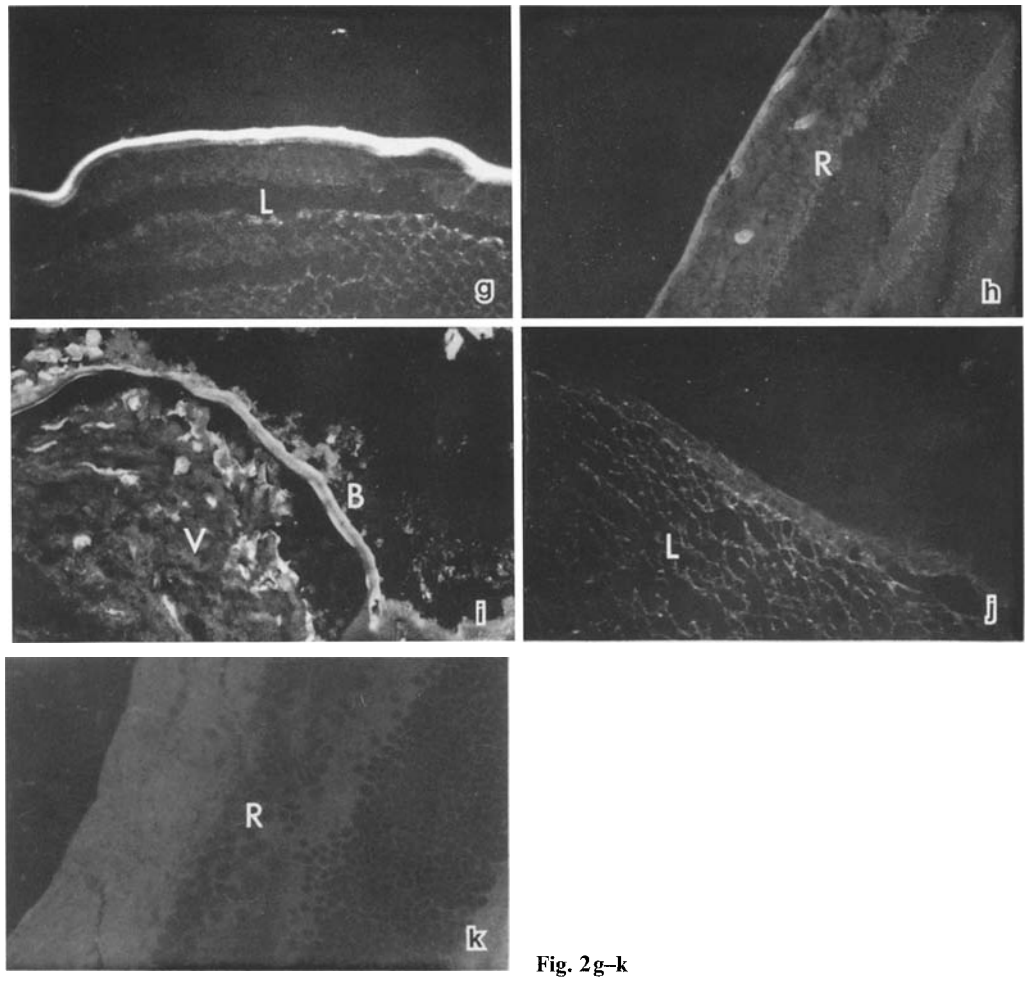

Fig. 2g–k

Table 1. IF staining and EM appearance of basement membranes (BM) in skin, lung, choroid plexus, lens, retina, and inner ear of unaffected, affected male, and carrier female dogs[a]

Dogs	Number	Tissue	Staining of BM by IF for				EM appearance of BM
			laminin	collagen type IV	fibronectin	GPA[b]	
Unaffected	2M, 5F	Skin	+	+	+	−	Normal epithelial, vascular, and neural BM
	5M, 5F	Lung	+	+	+	−	Normal epithelial and vascular BM
	1M, 4F	Choroid plexus	+	+	+	−	
	1M, 4F	Lens	+	+	−	+	Normal multilayered lens capsule
	1M, 1F	Retina	+	+	−	+	Normal internal limiting membrane
	2M	Inner ear	+	+	+	+[c]	Normal stria vascularis and basilar membrane
Affected male	4	Skin	+	+	+	−	Similar to those in unaffected dogs
	5	Lung	+	+	+	−	
	3	Choroid plexus	+	+	+	−	
	4	Lens	+	+	−	−	
	2	Retina	+	+	−	−	
	5	Inner ear	+	+	+	−	
Carrier female	2	Skin	+	+	+	−	Similar to those in unaffected dogs
	1	Lung	+	+	+	−	
	1	Choroid plexus	+	+	+	−	
	2	Retina	+	+	−	+	
	1	Lens	+	+	−	+	
	2	Inner ear	+	+	+	+	

[a] The BM examined in the various tissues are given in Materials and methods
[b] Goodpasture antigen
[c] Staining for GPA was seen in the basilar membrane but not in the stria vascularis

heads. Slit lamp illumination showed no lens abnormalities and no lesions were detected by ophthalmoscopic examination.

Hearing studies

All unaffected, affected male, and carrier female dogs made orientation responses to a dog whistle, confirming that they possessed some degree of hearing. In addition, auditory brain stem evoked responses did not reveal any differences in threshold levels or changes above 32 kHz between the unaffected and affected male dogs.

Discussion

Patients with HN may have a number of extra-renal manifestations, in addition to an inherited glomerulopathy which progresses to renal failure (Grünfeld 1985; Gubler et al. 1981; Habib et al. 1982; Nielsen 1978; Perrin et al. 1980). These include a sensori-neural hearing loss, initially involving the high frequency range, but eventually progressing to other frequencies and ultimately severe deafness. The incidence may be as high as 60%. In addition, there may be eye abnormalities in HN, such as anterior lenticonus (i.e., bulging of the lens into the anterior chamber of the eye) and macular changes. Lenticonus in HN is estimated to be present in up to 20% of kindreds. Macular changes, including perimacular pigmentation and loss of foveolar reflex, have been seen in up to 35% of male patients with HN and frequently accompany anterior lenticonus. However, they do not produce any deterioration in visual acuity, although they are associated with a poor renal prognosis.

The pathology of BM in extra-renal tissues in human HN has been poorly documented. A number of reports have described changes in the inner ear (Crawfurd and Toghill 1968; Gregg and Becker 1963; Myers and Tyler 1972; Weidauer and Arnold 1976), such as degeneration and atrophy of the stria vascularis; vacuolation of the spiral ligament; degeneration of sensori-neural structures, including the organ of Corti and spiral ganglion; foam cells in the endolymphatic sac; and hyalinization and atrophy of the tectorial membrane. The inner ear of a patient with Alport's syndrome has been examined post-mortem by one group of workers using EM, and blood vessels in the stria vascularis were interpreted to show changes similar to the lesion of GCBM (Weidauer and Arnold 1976). In addition, little is known about the eye in human HN (Nielsen 1978; Perrin et al. 1980; Gregg and Becker 1963). Thinning of the lens capsule leading to anterior lenticonus has been demonstrated in some studies using LM, but in others, only degeneration of the anterior lens fibers has been detected and there has been no mention of the lens capsule. Moreover, microscopy of the perimacular changes in the eye in human HN has not been performed, although the lesion is felt to be in the superficial layers of the retina, near the internal limiting membrane. However, EM studies have not been reported. Finally, a few studies have been reported in which EM was used to examine the skin of patients with HN. One mentions splitting of the epidermal-dermal and perivascular BM in the members of one family (Martinez-Hernandez and Amenta 1983), but other studies found no changes (Gubler et al. 1981; Kashtan et al. 1986). To our knowledge, no other tissue has been examined by EM in human HN.

The first main finding of our study relates to the appearance of extra-renal BM in the family of dogs with SHG that we studied. No differences were identified by LM and EM in BM of skin, lung, choroid plexus, lens, retina, or inner ear of affected male, carrier female or unaffected dogs; in particular, none of these BM showed the multilaminar splitting seen in GCBM of affected male dogs (Jansen et al. 1986a).

The second main finding of our study relates to the composition of extra-renal BM in SHG, as determined by IF. BM have been shown by IF to be composed of a mixture of collagenous and non-collagenous components (Martinez-Hernandez and Amenta 1983), including collagen type IV, laminin, heparan sulphate proteoglycan (HSPG), entactin, nidogen, and possibly collagen type V, fibronectin, and amyloid P protein. The presence of some of these components in glomeruli of patients with HN has been studied by IF. Collagen types IV and V, laminin, HSPG, and fibronectin have been demonstrated (Habib et al. 1982). Previously, we observed that collagen type IV, laminin, and fibronectin were also present in glomeruli of male dogs affected with SHG, as well as carrier female and unaffected dogs (Thorner et al. 1987). In the present study, we found that collagen type IV and laminin could be detected in BM of the skin, lung, choroid plexus, lens, retina, and inner ear of affected male, carrier female, and unaffected dogs. The tectorial membrane of the inner ear did not contain laminin or collagen type IV, and based on these and the EM results, this structure was not felt to be a BM. Fibronectin was not detected in the lens capsule or internal limiting membrane of the retina of any of the dogs, although it was present in BM of the other tissues.

The third main finding of our study concerns the detection of GPA by IF in the extra-renal BM of the dogs. We failed to detect GPA in BM of the skin, lung, choroid plexus, or stria vascularis of the inner ear in any of the dogs, even after the tissue sections had first been treated with acid urea to expose GPA. In contrast to the foregoing negative results, GPA was detected in the lens capsule, internal limiting membrane of the retina, and basilar membrane of the inner ear of unaffected and carrier female dogs.

The presence of GPA in normal human tissues has been determined by routine IF in a number of studies, using eluates of kidney tissue from patients with Goodpasture syndrome, serum from patients with Goodpasture syndrome, and monoclonal antibodies. GPA has been demonstrated consistently in GCBM, occasionally in BM of lung and choroid plexus, lens capsule, internal limiting membrane of the retina, cochlea, and tectorial membrane of the inner ear, and not at all in BM of heart, liver, gastro-intestinal tract, spleen, adrenal gland, skeletal muscle, or skin (Lockwood 1984; McIntosh et al. 1975; McPhaul and Dixon 1970; Pressey et al. 1983; Pusey et al. 1987; Savage et al. 1985; Savage et al. 1986; Wick and Timpl 1980; Wieslander et al. 1984a). Detection of GPA in some but not all BM may have several explanations. First, there may be heterogeneity of the Goodpasture sera used to detect GPA in different studies, leading to different results by IF even though similar tissues were examined. This possibility was emphasized in our previous study in which only one of 10 such sera tested was shown to cross-react with dog GCBM (Thorner et al. 1987). Second, BM may differ in various sites, so that some possess GPA in an exposed form while others possess it in a masked form or not at all. The possibility of masking was supported by the observation that treatment of tissue sections of human skin, lung, and placenta with acid urea before applying Goodpasture serum and fluoresceinated anti-human immunoglobulin led to staining for GPA in BM of these tissues, which was not observed without acid urea treatment. These results suggested that GPA was a ubiquitous component of BM. However, it was also reported that not all BM stained for GPA following acid urea (Yoshioka et al. 1985), suggesting that the Goodpasture sera used in these studies were also heterogeneous. Third, if GPA is a ubiquitous component of BM, there may be differences in the antigenicity of GPA in different BM. There is indirect evidence to support this view. First, GPA which was isolated from BM of human lung and placenta showed reactivity with Goodpasture serum of only 40% and 10% respectively compared with GPA isolated from kidney (Wieslander and Heinegård 1985). Second, although gel electrophoresis of GPA revealed bands of 26K and 50K in all 3 tissues (Wieslander et al. 1984a, b; Wieslander and Heinegård 1985), immunoblotting using Goodpasture serum showed binding to only the 50K band of the GPA isolated from lung and placenta (Wieslander and Heinegård 1985), but binding to both the 50K and 26K bands in the case of GPA isolated from kidney (Wieslander et al. 1984a, b). We postulate that the Goodpasture serum used in our study was directed against antigenic sites on GPA which were present only in GCBM, lens capsule, internal limiting membrane of the retina, and basilar membrane of the inner ear, accounting for the failure to detect GPA in BM of skin, lung, and choroid plexus, even after acid urea treatment.

GPA has been shown to correspond to the C-terminal region of the collagen type IV molecule, referred to as the NC1 domain (Wieslander et al. 1984a, b; Timpl et al. 1981; von der Mark et al. 1985). There may be several explanations as to why GPA may not be identical in BM in all tissues. First, there is evidence that NC1 exists in a precursor form, from which a 2K fragment is removed before collagen type IV is secreted from the cell (Timpl et al. 1985). Variations in the NC1 domain may result from differences in proteolytic cleavage, leading to differences in GPA in various BM. Second, variation in the structure of GPA may arise if there are different subtypes of the collagen type IV molecule or different proportions of the α-chains of collagen type IV in different tissues (Wieslander and Heinegård 1985). There is precedence for the existence of different chains in the case of collagen type V, which exists in two forms, one composed of $\alpha 1$(V) and $\alpha 2$(V) chains and the other of $\alpha 1$(V), $\alpha 2$(V), and $\alpha 3$(V) chains (Kumamoto and Fessler 1980; Sage and Bornstein 1979). Collagen type IV is generally felt to be derived from $\alpha 1$(IV) and $\alpha 2$(IV) chains in a ratio of 2:1 (Fessler and Fessler 1982; Crouch et al. 1980; Dixit 1979), but some evidence points to the existence of a third type of chain, namely $\alpha 3$(IV) (Dixit et al. 1985; Timpl and Martin 1982). Although the NC1 region is generally considered to be composed of monomers derived from $\alpha 1$(IV) and $\alpha 2$(IV) chains, some workers have detected a third monomer, the origin of which is not clear (Butkowski et al. 1985; Wieslander et al. 1985). Further work is needed to determine whether this third monomer is an $\alpha 3$(IV) chain.

The fourth main finding of our study relates

to our inability to detect GPA in GCBM (Thorner et al. 1987) or any extra-renal BM of affected male dogs. Similarly, in human patients with HN, Goodpasture serum has failed to stain GCBM by IF in most affected males and some affected females, suggesting that there is an absence of GPA in their GCBM (Jenis et al. 1981; Jeraj et al. 1983; McCoy et al. 1982; Olson et al. 1980). A similar result has been obtained using a monoclonal antibody to GPA (Savage et al. 1986). In addition, one previous study utilized IF and a human serum which was obtained from a HN patient who had received a renal transplant and then developed an anti-GCBM antibody-mediated glomerulonephritis in the transplant (Kashtan et al. 1986). Affected males with HN failed to show positive staining of BM at the epidermal-dermal junction of skin, while most carrier females showed interrupted linear staining. Our inability to detect GPA by IF in affected male dogs implies either that GPA is not present at all or that its antigenicity has been altered. Nevertheless, no abnormalities were detectable by EM, nor were there any clinical abnormalities of vision or hearing. Thus, it would appear that maintenance of the integrity of these BM in affected male dogs did not require intact GPA, in contrast to GCBM, which showed multilaminar splitting if GPA was absent (Jansen et al. 1986a). The reason for this difference in these various tissues is not known but it is possible that there is an additional factor which operates in the kidney to produce an abnormal GCBM but not in the lens, retina, or inner ear. We postulate that GCBM are exposed to a higher tension from the circulation of the blood than these other BM, which are not directly exposed to the circulation, making GCBM less able to tolerate any defects in GPA or the NC1 domain.

Many but not all patients with HN are afflicted by a combination of kidney, eye, and ear disease (Grünfeld 1985; Gubler et al. 1981; Habib et al. 1982). Why extra-renal manifestations do not occur in all patients is not known. As postulated in dogs, there may be some superimposed factor in human HN which brings out an otherwise latent abnormality. A second possibility is that HN is actually a group of diseases, resulting from more than one gene defect. Insight into the pathogenesis of the eye and ear lesions in some patients with HN may be obtained by extrapolating to man the finding that GPA is absent from the lens capsule, internal limiting membrane of the retina, and basilar membrane of the inner ear, as well as GCBM (Thorner et al. 1987), of affected male dogs with SHG. Hence, it is reasonable to implicate a defect in GPA (i.e., NC1 domain) in the development of these extra-renal lesions in man. The NC1 domain functions as a cross-linking component of the collagen type IV molecule (Wieslander et al. 1984a; von der Mark et al. 1985; Bächinger et al. 1982; Weber et al. 1984; Yurchenco et al. 1986), and therefore an abnormality in its structure could lead to weakened BM, manifested in the kidney as multilaminar splitting of GCBM. Since the lens capsule is under constant tension produced by the lens fibers, anterior lenticonus could result if the capsule were weakened. The basilar membrane of the inner ear requires both strength and elasticity during its displacement in response to sound waves. A weakness in this structure could impair this function, resulting in sensori-neural hearing loss. Since higher frequency sound vibrations carry greater energy, they may produce greater stress on the basilar membrane; thus any weakness in it might be manifested first in the high frequency range. Further characterization of the gene defect in SHG may lead to a better understanding of the functional domains of the NC1 region, including GPA, and provide greater insight into subtypes of human HN and possible variations in BM structure in different organs.

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