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Päivi Ljungberg · Ismo Virtanen · Christer Holmberg
Hannu Jalanko

Distribution of renal integrin receptors and their ligands in congenital nephrotic syndrome of the Finnish type

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Abstract The aim of this study was to examine the distribution of β1 and αv integrins (Ints) and some of their ligands in the kidneys of patients with congenital nephrotic syndrome of the Finnish type (CNF) and in controls using indirect immunofluorescence with monoclonal antibodies. The mesangial reactivity of Int α1 and Int β1 subunits was more variable and an increased glomerular reactivity with Int α3 and Int-α6 antibodies was found in CNF kidneys than in controls. Int α2 subunit was either completely missing from or found in significantly lesser amounts in CNF kidney glomeruli. The immunoreactivity for Int αv was more variable, fainter and also more granular in CNF samples than in control kidneys. The glomerular reactivity for Int β5 was more diffuse and weaker, and in sclerotic Bowman's capsules more intense in CNF kidneys than in controls. Immunoreactivity for Int β6 was restricted and was comparable in extent in CNF and control kidneys. Of the extracellular matrix components studied, the expression of EDAFn, EDBFn, OncFn, Ln α2 chain, Ln β1 chain and tenascin was increased. This is also seen in several glomerular diseases with inflammation and sclerosis. Immunoreactivity for vitronectin was decreased. Several differences were found in the intensity or location of the immunostaining for the β1 and αv Ints and their ligands in CNF kidneys compared with controls, which have not been found in any other proteinuric disease. Disturbed Int expression pattern in CNF may specifically reflect the disturbance of glomerular function caused by the primary defect in this disease.

P. Ljungberg (✉)
Department of Bacteriology and Immunology,
Haartman Institute, University of Helsinki,
P.O. Box 21 (Haartmaninkatu 3),
FIN-00014 Helsinki, Finland
Tel.: (358) 90–434 6368, Fax: (358) 0–434 6382

P. Ljungberg · C. Holmberg · H. Jalanko
Children's Hospital, Helsinki University,
Central Hospital, Helsinki, Finland

I. Virtanen
Institute of Biomedicine, Department of Anatomy,
University of Helsinki, Helsinki, Finland

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Introduction

Congenital nephrotic syndrome of the Finnish type (CNF) is an autosomal recessive disease that occurs throughout the world but is more frequent in Finland than in other countries. CNF presents typically with heavy proteinuria starting in intrauterine life. There is prematurity, and the nephrotic syndrome develops within the first weeks of life. Nowadays this previously fatal disease is treated by early renal transplantation [16].

The renal pathology in CNF has been studied by several investigators, but the basic defect has remained elusive [34]. In electron microscopy, spreading of podocyte foot processes and thinning of lamina densa of the glomerular basement membrane (GBM) can be detected [3, 34]. Decrease of anionic charge and, especially, the decreased amount of heparan sulfate proteoglycan in the GBM are considered to be the pathogenetic mechanism in other congenital nephrotic syndromes (CNS) [40, 41], but no such decrease has been found in CNF [26, 28, 39]. The faulty synthesis of some other GBM components has also been thought to be responsible for the abnormal proteinuria in CNF [35]. Recently Kestilä et al. [20] showed that at least eight genes coding for the GBM components are intact in CNF. The presumptive CNF locus has been mapped to the long arm of chromosome 19 on the basis of linkage analyses in 17 Finnish CNF families. This region of the chromosome 19 does not contain any potential candidate genes coding for known molecular components of the kidney [19].

The integrin (Int) family of extracellular matrix (ECM) receptors mediate cellular attachment to ECM and GBM and are also important modulators of cellular function [17]. The β1 Ints have been shown to have a cell specific and characteristic expression pattern during nephrogenesis as well as in adult human kidneys [5, 22, 23, 31, 36]. In addition, the potential involvement of Int

Table 1 Earlier characterised monoclonal antibodies (MAbs) recognising integrin subunits and extracellular matrix components used in this study

MAb recognised	Structure	Reference
102DF7	Int β1	Ylänne and Virtanen, 1989
PL 90BB10	Int β3	Ylänne et al., 1988
AA3	Int β4	Tamura et al., 1990
1A9	Int β5	Pasqualini et al., 1993
E7P6	Int β6	Weinacker et al., 1994
TS2/7	Int α1	Hemler et al., 1984
CLB10G11	Int α2	Giltay et al., 1989
J143	Int α3	Hemler et al., 1987a
B5G10	Int α4	Hemler et al., 1987b
B1E5	Int α5	Werb et al., 1989
GoH3	Int α6	Sonnenberg et al., 1987
52DH1	EDAcFn	Vartio et al., 1987
BC-1	EDBcFn	Carnemolla et al., 1989
FDC-6	OncFn	Matsuura et al., 1988
100EB2	Tenascin	Howeedy et al., 1990
4C7	Laminin α1 chain	Engvall et al., 1990
5H2	Laminin α2 chain	-"-
BM2	Laminin α3 chain	Marinkovich et al., 1992
4E10	Laminin β1 chain	Engvall et al., 1990
C4	Laminin β2 chain	Hunter et al., 1989
2C8	Laminin γ1 chain	Engvall et al., 1990

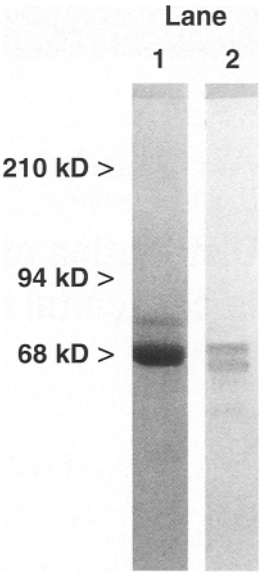


Fig. 1 Characterisation of the monoclonal VN2-antibody-detecting vitronectin. *Lane 1* shows purifired vitronectin in SDS-PAGE with amidoblack staining. *Lane 2* shows the same material immunoblotted with VN2-antibody revealing two separate bands

receptors in glomerular injury and proteinuria has recently been postulated [1, 7, 9]. The information on integrin expression in human glomerular diseases is scanty, however, and no studies have been performed on the integrin distribution in congenital nephroses.

The aim of this study was to examine the distribution of β1 and αv Ints, the cellular receptors for GBM components, and some of their more recently characterised ligands in the kidneys of CNF patients. Since this syndrome is a disease with primary proteinuria, it could provide further information on the function of integrins in kidneys.

Methods

The diagnosis of CNF was made on the basis of typical clinical features, kidney histology and the exclusion of other types of congenital nephrotic syndrome (CNS) [34]. The kidneys were obtained by nephrectomy performed in the course of treatment [16]. Normal parts of kidneys removed for renal cancer were used as controls. The tissues were immediately frozen in either melting freon or isopenthane cooled in liquid nitrogen, or directly in liquid nitrogen, and stored at –70°C until used. Samples from five CNF patients were compared with those from two controls.

Table 2 Location of Int-β and Int-α subunits in CNF and control kidneys. – Negative, (+) very weak, + weak, ++ strong, ? questionable immunoreactivity

Int-β-subunits	β1		β3		β5		β6	
	CTRL	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*
Glomerulus								
Parietal ep.	++	++	(+)	(+)/+	-/(+)	-/+	-	-
Visceral ep.	++	++	(+)	-/(+)	+	-/(+)	-	-
Endothelium	++	++	(+)	-/(+)	+	-/(+)	-	-
Mesangium	++	+/++	-	-	-	-	-	-
Tubular cells	++	++	(+)/+	(+)/+	+	+	++	++

Int-α-subunits	α1		α2		α3		α6		αv	
	CTRL	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*
Glomerulus										
Parietal ep.	+	+/++	-	-/+	++	++	-/+	-/+	+	-/++
Visceral ep.	-	-	-	-	++	++	-	-/+	+	-/+
Endothelium	+	+	-	-	-	++	+	+/++	-	-
Mesangium	+	+/++	+	-/+	-	?	-	-	-	-/+
Tubular cells	+	+	+	+	+/++	+/++	++	++	+	-/++

Monoclonal antibodies (mAbs) against Int subunits and extracellular matrix components are presented in Table 1. Mab AA3 was obtained from V. Quaranta, Scripps Clinical Research Foundation, San Diego, Calif., Mab 1A9, from M. Hemler, Dana Farbor Cancer Institute, Boston, Mass., Mab E7P6 from D. Sheppard, Department of Medicine, University of California, San Fransisco, Calif., J143 from L. J. Old, Ludwig Institute of Cancer Research, New York, N.Y., Mab B5G10 from M. Hemler, Mab B1E5 from C. Damsky, BC-1 from Prof. L. Zardi, Inst. Ric. Cancro, Department of Cell Biology, Genova, Italy, FDC-6 from Prof. S. I. Hakomori, Biomembrane Institute, Seattle, Wash. MAb 100EB2 and Mab 4C7, Mab 4E10, Mab 2C8, Mab 5H2, Mab C4 were obtained from Dr. E. Engvall (Wennor Gren Institute, Stockholm, Sweden) and J. Sanes (Washington University, St. Louis, Mo.), respectively. The Mab BM2 was obtained from Dr. R. E. Burgeson (Harvard University Cutaneous Research Center, Boston, Mass.). The MAb against talin was obtained from Serotec (Kidlington, Oxford, England) and that against focal adhesion kinase, pp125^{FAK}, from Dr. J. T. Parsons (University of Virginia, Charlottesville, Va.).

The MAb VN2 was raised against vitronectin isolated from outdated human plasma (Finnish Red Cross Blood Transfusion Service, Helsinki, Finland) by using affinity chromatography as described by Yatogho et al. [45]. For this purpose, 50 µg of protein was used to immunise BALB/c mice. Standard methods of monoclonal antibody production were used [21]. Cloning of the positive hybridomas was done manually by picking up single cells. One of the clones (VN2) was characterised further and was found to detect in Western blotting of SDS-PAGE-separated vitronectin its major M_r 75 000 polypeptide as well as the smaller modified subunit (Fig. 1, lane 2) although it was seen as a single band in amidoblack staining (Fig. 1, lane 1).

For indirect immunofluorescence frozen sections 5 µm thick were cut and fixed in acetone cooled to –20°C, for 5 min. The sections were incubated with mAbs for 30 min, and subsequently with fluorescein isothiocyanate (FITC)-coupled goat anti-mouse antiserum (Jackson Laboratories, West Grove, Pa.) for 30 min. The specimens were then mounted in buffered glycerol. In control experiments the primary mAb was omitted or replaced with an irrelevant mAb produced by one of us (I.V.). The samples were examined with a Zeiss standard microscope equipped with a filter system for FITC fluorescence and phase-contrast optics.

Results

The comparison of the location and intensity of the immunoreactivity for monoclonal antibodies detecting integrins and extracellular matrix components is presented in Tables 2 and 3. The most pronounced phenomena were as follows.

Both normal and CNF glomeruli showed a capillary loop type of reactivity for *Int β1*, as described earlier [23]. The mesangial immunoreactivity of CNF glomeruli varied more within and between glomeruli (Fig. 2B, D) than did that in normal kidney glomeruli (Fig. 2A). The immunoreactivity for talin followed that of Int β1 in both control and CNF kidneys (Fig. 2C, E). No immunoreactivity for pp125^{FAK} (focal adhesion kinase) could be detected in either control or CNF kidneys (data not shown).

For *Int β3,* in some CNF kidneys the glomerular and tubular reactivities were weaker (Fig. 3B) than in controls (Fig. 3A). The reactivity with *Int β4* antibodies was comparable in control and CNF kidneys: an extremely weak reactivity was found in the basal aspects of collecting ducts (data not shown).

Table 3 The distribution of extracellular matrix components in CNF kidneys as compared to controls. – Negative, + weak, ++ strong immunoreactivity. *EDAFN* EDA domain of fibronectin, *EDBFN* EDB domain of fibronectin, *oncFN* oncofetal fibronectin, *Lnα1* α1-chain of laminin, *Lnα2* α2 chain of laminin, *Lnβ1* β1-chain of laminin, *Lnβ2* β2-chain of laminin

	EDAFN		EDBFN		oncFN		Tenascin		Vitronectin		Ln α1		Ln α2		Ln β1		Ln β2	
	CRTL	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*	*CTRL*	*CNF*
Glomerulus																		
GBM	-	-/+	-	-/+	-	-/++	-	-	++	-/+	++	++	-	-/+	-	-	++	++
Mesangium	+	+/++	-	-/+	-	-/++	++	++	++	-/+	-	-	+	-/++	+	-/++	-	-
BC	-	-/++	-	-/+	-	-/++	++	++	-/+	-/+	+	+	-/++	-/++	++	++	-	-
Tubular BM	-	-	-	-	-	-	++	++	++	-/+	++	++	-	-	++	++	-	-
Interstitium	+	++	-	-/+	-	-/++	-	++	-	-	-	-	-	+	-	-	-	-

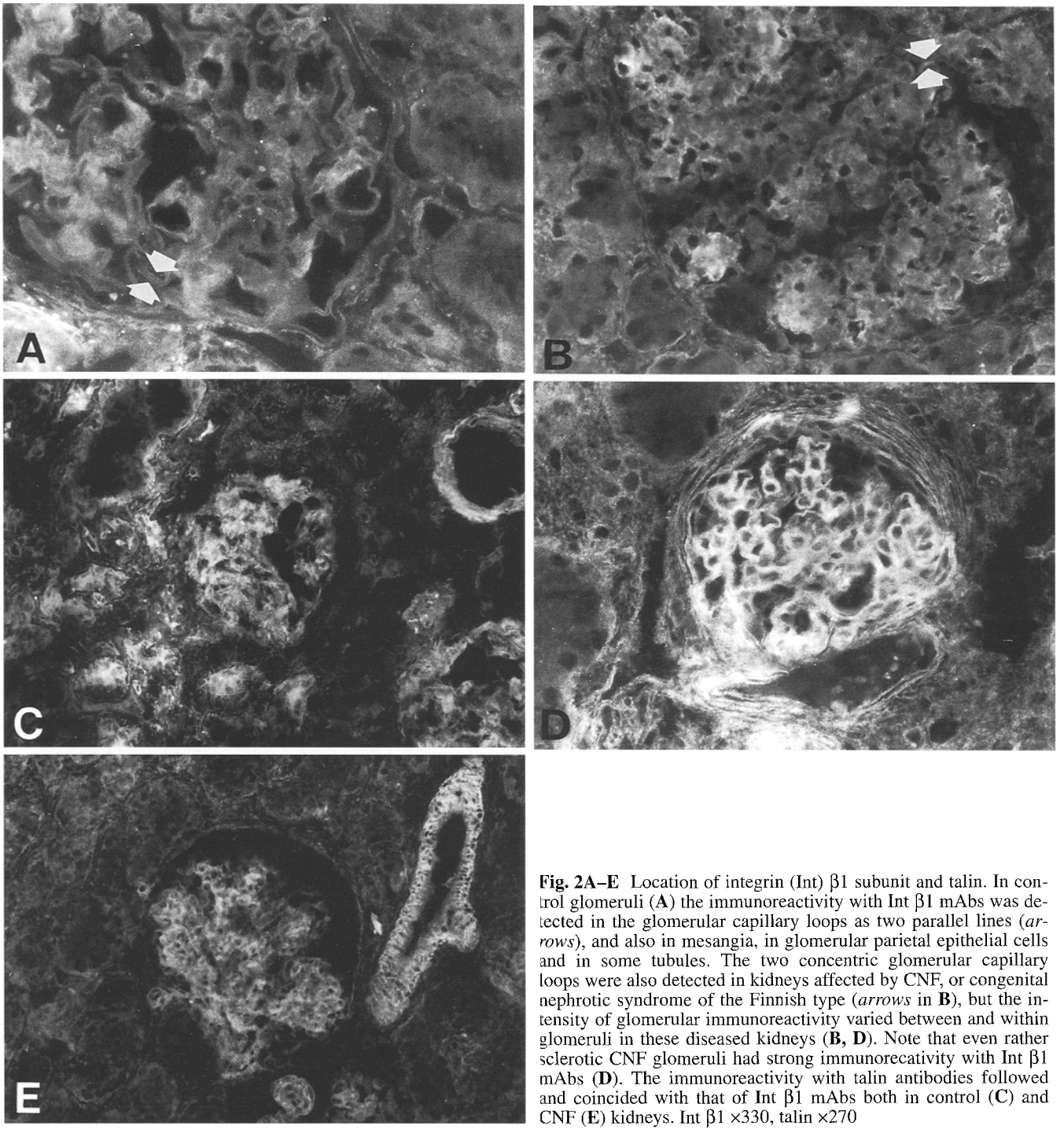


Fig. 2A–E Location of integrin (Int) β1 subunit and talin. In control glomeruli (**A**) the immunoreactivity with Int β1 mAbs was detected in the glomerular capillary loops as two parallel lines (*arrows*), and also in mesangia, in glomerular parietal epithelial cells and in some tubules. The two concentric glomerular capillary loops were also detected in kidneys affected by CNF, or congenital nephrotic syndrome of the Finnish type (*arrows* in **B**), but the intensity of glomerular immunoreactivity varied between and within glomeruli in these diseased kidneys (**B, D**). Note that even rather sclerotic CNF glomeruli had strong immunorecativity with Int β1 mAbs (**D**). The immunoreactivity with talin antibodies followed and coincided with that of Int β1 mAbs both in control (**C**) and CNF (**E**) kidneys. Int β1 ×330, talin ×270

The immunoreactivity for *Int β5* localised with moderate intensity in the glomerular mesangium and in a capillary-loop-like distribution, notably more strongly on the podocyte side of the loop. It was also seen locally in some Bowman's capsules in control kidneys (Fig. 3C). The mesangial reactivity was sometimes granular in pattern in control kidney glomeruli. Most tubuli reacted with moderate intensity in a basally polarised manner and some also in an overall pattern in controls. In CNF kidneys the glomerular reactivity seemed more diffuse and in some CNF samples was also weaker than in controls (Fig. 3D). The sclerotic Bowman's capsules in CNF kidneys were strongly positive, while others were locally positive, as in controls. The tubular reactivity in nephrotic kidneys was comparable to than in controls.

The monoclonal antibodies detecting *Int β6* subunit reacted diffusely with epithelial cells of some rare tubules in normal kidneys (Fig. 3E, F). The tubular reactivity was often granular in pattern. The glomeruli were al-

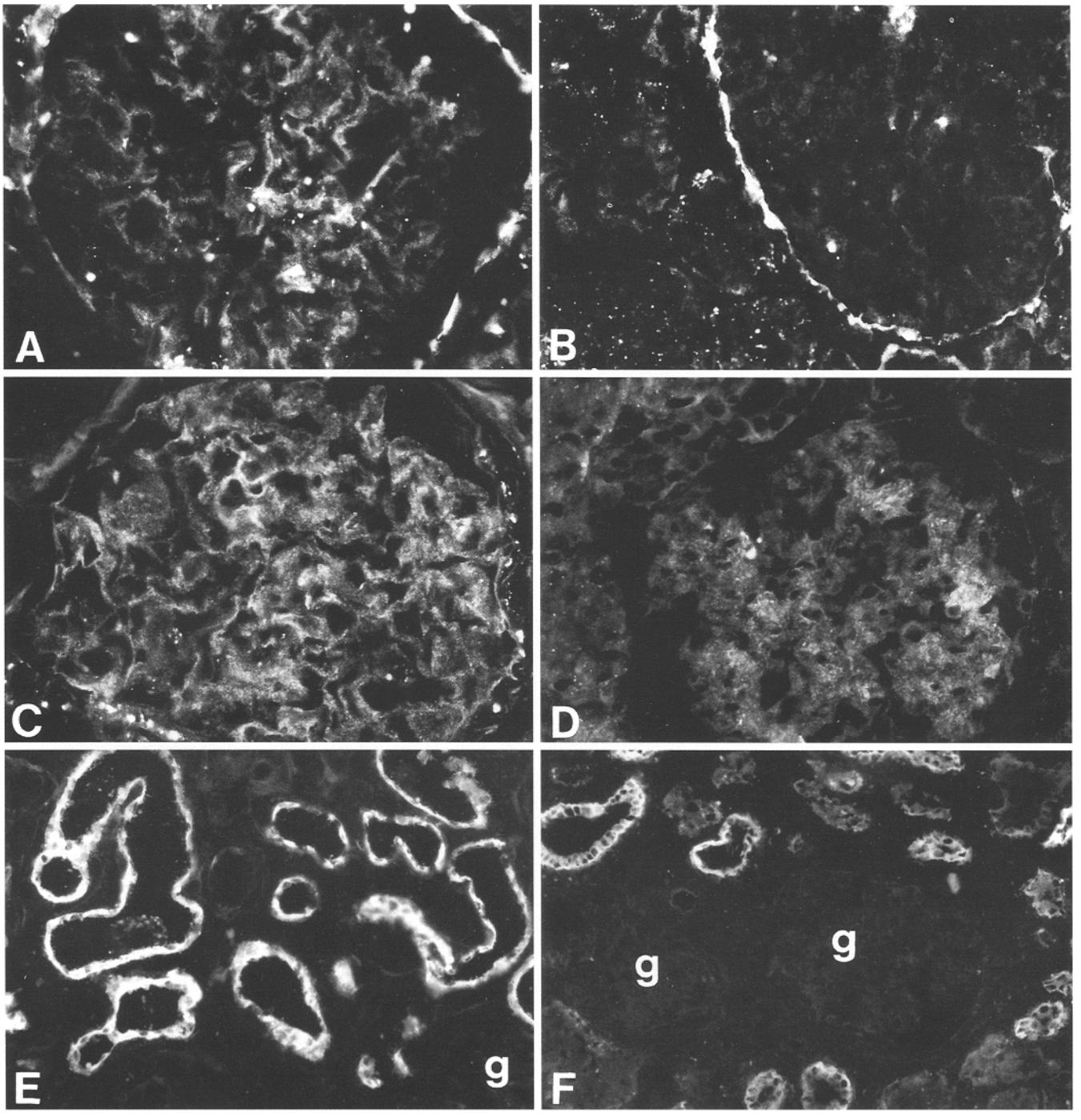
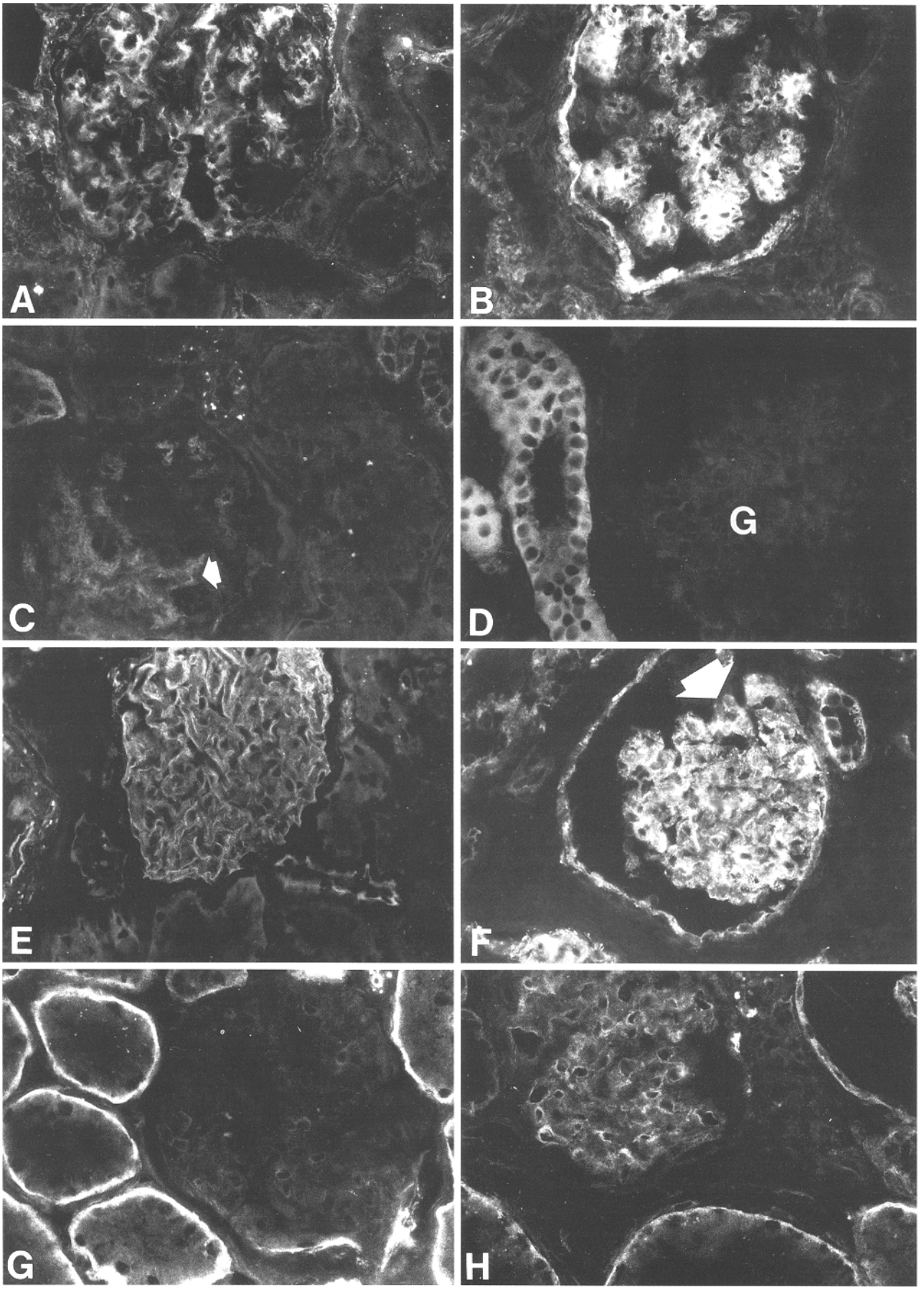


Fig. 3A–F The location of Int β3, Int β5 and Int β6 subunits. Int β3 is located in glomerular podocytes abutting the GBM and in endothelia as well as in parietal epithelial cells in control kidneys (**A**). This distribution of Int β3 cannot be found in CNF glomeruli, where the glomerular immunoreactivity with MAbs detecting this subunit is clearly weaker than in controls (**B**). Note, however, the bright immunofluorescence with Int β3 antibodies in the parietal epithelium in CNF glomeruli. The glomerular immunoreactivity of Int β5 subunit was weaker and more diffuse in CNF (**D**) than in control (**C**) kidneys. The overall immunoreactivity with Int β6 mAbs in some, rare, tubuli was detected similarly both in CNF (**F**) and control (**E**) kidneys (negative glomeruli are shown with *g*). Int β3, Int β5 ×670, Int β6 ×270

most negative; some extremely faint, possibly capillary-loop-like, reactivity was, however, found. In CNF kidneys the reactivity was similar to that seen in control kidneys.

For *α1 Int* the immunoreactivity of mesangial areas, as well as Bowman's capsules, was more variable in CNF kidney glomeruli (Fig. 4B) than in controls (Fig. 4A). The *α2 Int subunit* detected with monoclonal antibodies was localised in glomerular mesangium and in some tubuli in the normal kidneys [22] (Fig. 4C). In CNF kidneys no mesangial reactivity could be detected or the reactivity found in some samples was clearly


A
B
C
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E
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G
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weaker than in controls (Fig. 4D). Some Bowman's capsules had an extremely weak and uneven staining pattern in the CNF kidney sections. The tubules reactive with the α2 Int antibodies included dilated ones typical of CNF.

In the kidney sections of CNF patients, strong glomerular reactivity for *Int α3* was detected in a capillary-loop-like manner and also in mesangia (Fig. 4F), differing distinctly from that of controls (Fig. 4E).

Both control and CNF samples were negative with MAbs detecting *α4* and *α5 Ints* (data not shown). The glomerular immunostaining pattern for *α6 Int* of CNF kidneys (Fig. 4H) was distinctly different from that of control glomeruli (Fig. 4G): a strong, capillary-loop-like reactivity was detected in most glomeruli. There was, however, again a notable variability of intensity between and within different glomeruli in the diseased kidneys.

The *αv Int* was localised in the normal kidney glomeruli in a capillary-loop-like manner. The epithelial cells of Bowman's capsules and some tubules also revealed reactivity (Fig. 4A). The glomerular immunostaining in CNF samples was more granular, variable, and weaker than in the glomeruli of control kidneys (Fig. 4B). In some CNF samples the reactivity of tubuli and Bowman's capsules was also more granular and uneven, and occasionally also stronger, than in controls, while in others the reactivity was comparable to that of controls.

Expression of laminin (Ln) chains was examined. The immunoreactivity for *Ln α1 chain* was noted to be similar in control kidneys (as earlier described [42]) and in CNF kidneys (Fig. 5A, B). In CNF kidney glomeruli *Lnα2 chain* was occasionally found in the GBM in addition to mesangia, and Bowman's capsules in control kidneys (Fig. 5C), as described earlier [42]. The mesangial reactivity varied in CNF kidney sections: there were some totally negative glomeruli and also glomeruli with strong mesangial reactivity (Fig. 5D). In CNF kidneys Ln α2 chain was occasionally located in interstitial areas.

No immunoreactivity for the *Ln α3 chain* could be detected in either control or CNF kidneys (data not shown). In the location of *Ln β1 chain* the distinct difference noted in CNF kidneys compared with controls (Fig. 5E) was the variability in glomerular immunostaining. Some glomeruli in CNF samples were totally negative, as some revealed a strong mesangial reactivity (Fig. 5F).

The immunoreactivity for *Ln β2* was seen brightly in GBMs and the BMs of arteries, and weakly in interstitia in both normal human adult kidneys (as described earlier [42]) and CNF kidneys (Fig. 5G, H). The immunoreactivity for *Ln γ1* was detected faintly in all tubular and glomerular basement membranes, both in control (as described earlier [42]) and in CNF kidney sections (data not shown).

In CNF kidney glomeruli the mesangial reactivity for *extradomain A of cellular fibronectin (EDAFn)* varied between glomeruli, and a mostly increased reactivity was seen, compared with controls (Fig. 6A, B) [25]. In some, nephrotic glomeruli reactivity was also found in GBMs. The interstital immunoreactivity was significantly increased in the CNF kidney sections. In the nephrotic glomeruli some Bowman's capsules were negative and some, mainly those of sclerotic glomeruli, were strongly positive.

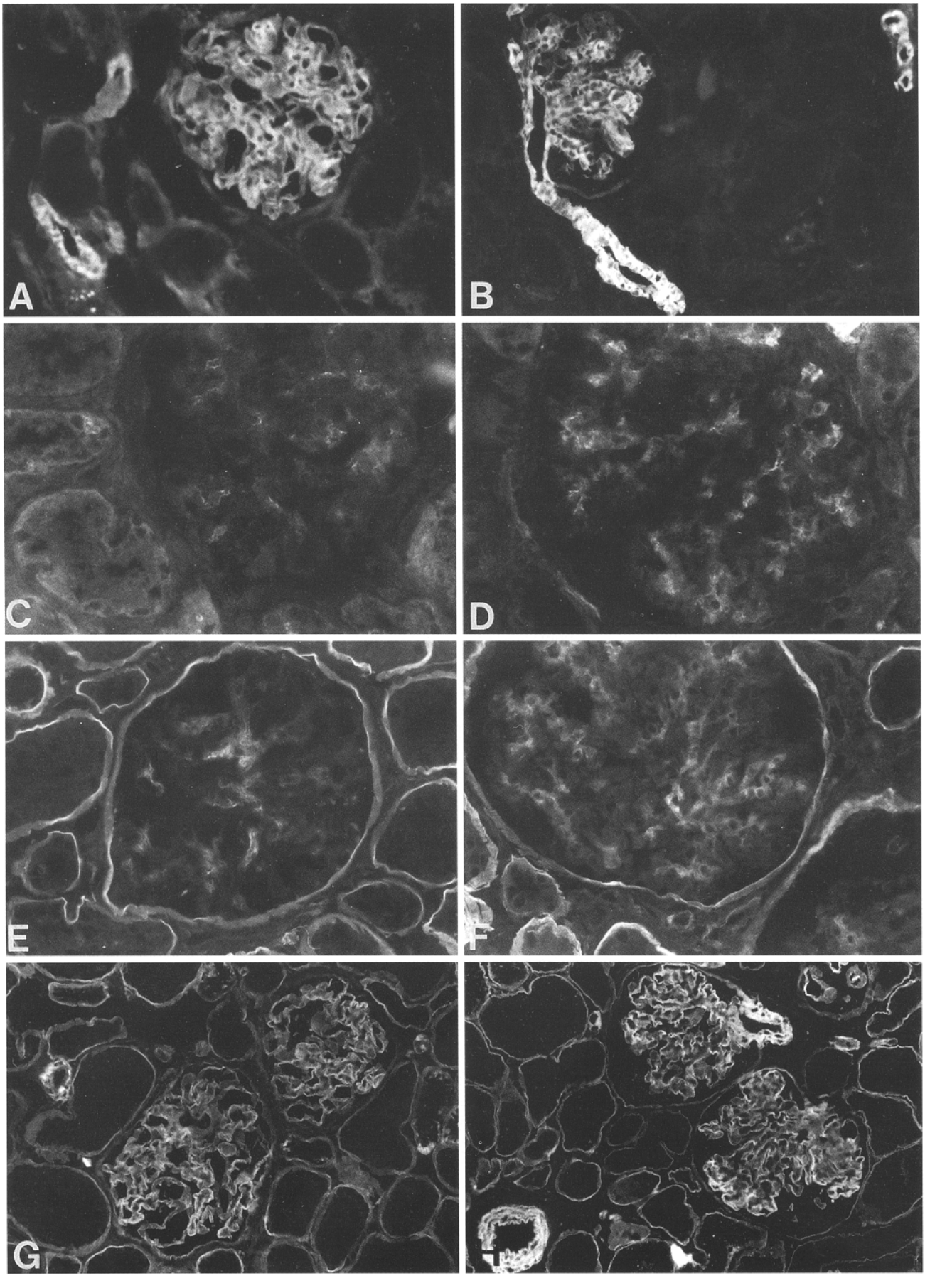
The normal kidneys revealed no reactivity with monoclonal antibodies detecting the *extradomain B of cellular fibronectin (EDBFn)* [25]. CNF kidneys revealed weak and uneven immunostaining, with some glomerular mesangia, Bowman's capsules and interstitial areas reacting with EDBFn antibodies. Thus, the immunostaining pattern of CNF kidney sections differed distinctly from that of controls (Fig. 6E).

The normal kidney sections were completely negative in anti-*oncFn (oncofetal form of cellular fibronectin)* immunostaining, as described earlier [25] (Fig. 6C). In CNF kidney sections a spotty, weak to moderate reactivity was detected in glomerular mesangia and possibly also BMs in interstitial areas (Fig. 6D). Only a few Bowman's capsules revealed moderate reactivity in CNF glomeruli.

Tenascin immunoreactivity was located in the mesangial areas of glomeruli, in Bowman's capsules, and in tubular basement membranes with strong intensity of immunoreactivity in normal kidneys as described earlier [25] (Fig. 7E). In CNF kidney sections the reactivity of interstitial areas was stronger (Fig. 7G), whereas the mesangial staining was somewhat stronger or comparable to that of controls (Fig. 7F).

In normal kidneys *vitronectin*, as detected with monoclonal antibodies, was located with moderate reactivity in the glomerular mesangium and BMs, in the BMs of blood vessels and tubules and also locally, in Bowman's capsules (Fig. 7C). In CNF kidney sections, the glomerular reactivity was weaker and more granular in pattern than in control sections (Fig. 7D). The tubular and Bowman's capsular immunostaining was more uneven than in controls. In some tubular cells granular reactivity was found in CNF kidneys.

Fig. 4A–H Distribution of Int α1, Int α2, Int α3 and Int α6. **B** shows a CNF glomerulus with stronger, especially mesangial, immunoreactivity with Int α1 antibodies than a glomerulus from a control kidney (**A**). The mesangially confined immunoreactivity of Int α2 in control glomeruli (*arrow* in **C**) cannot be found in a CNF glomerulus (**D**, negative glomerulus shown with **G**). In the glomeruli of control kidneys the immunoreactivity of Int α3 is restricted to the podocyte membranes abutting the glomerular basement membrane, or GBM (**E**), whereas in CNF glomeruli a distinctly stronger mesangial and capillary-loop-like reactivity is noted (**F**). Note also the bright immunofluorescence of parietal epithelial cells of CNF glomerulus (*arrow* in **F**). The intensity of glomerular immunoreactivity with Int α6 antibodies was also clearly stronger in CNF kidneys, being more like the capillary loop in pattern (**H**) than the endothelially restricted pattern detected in control kidneys (**G**). Note the similar, basally confined reactivity with Int α6 antibodies in both CNF and control tubules. ×330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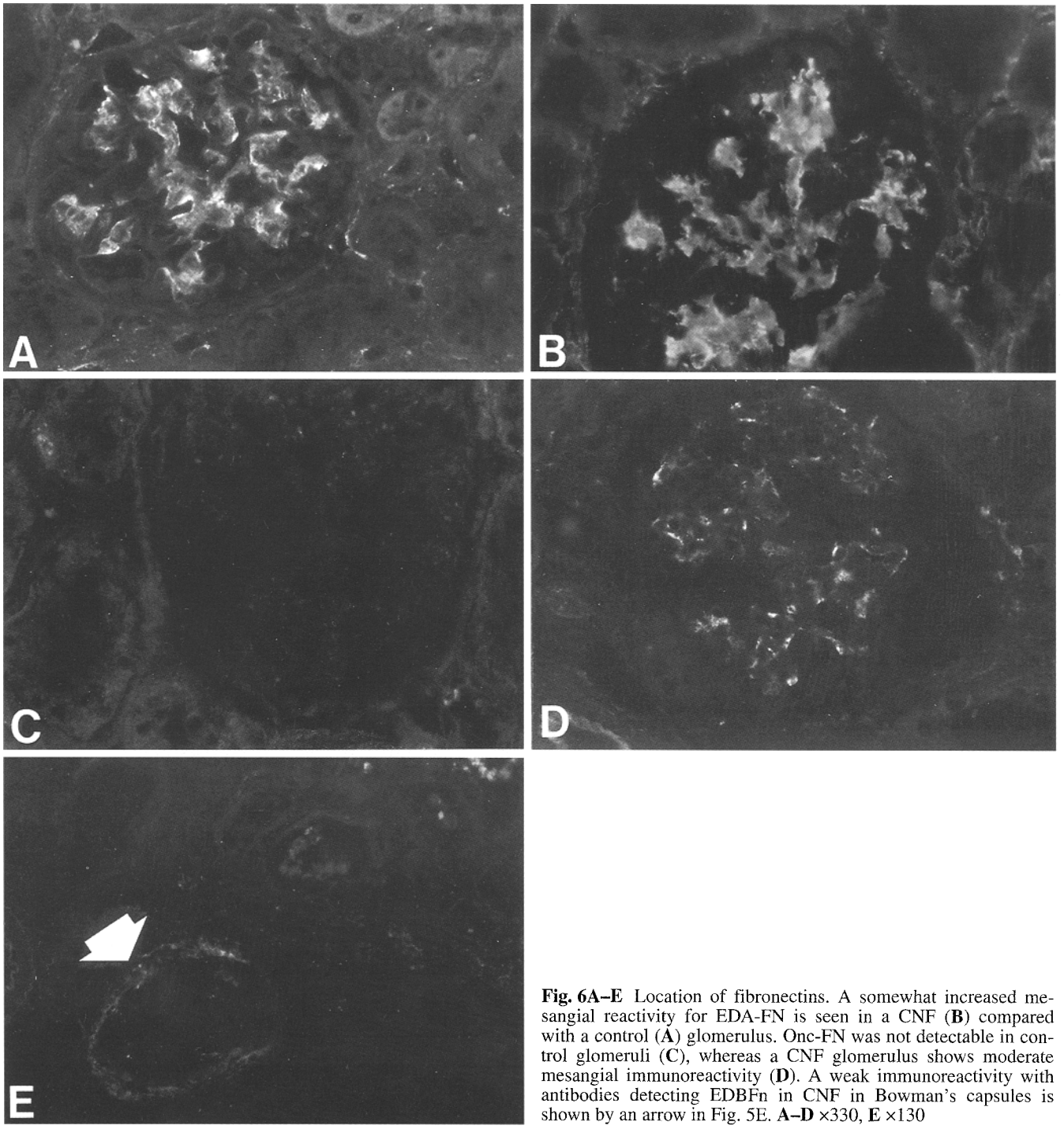

Fig. 6A–E Location of fibronectins. A somewhat increased mesangial reactivity for EDA-FN is seen in a CNF (**B**) compared with a control (**A**) glomerulus. Onc-FN was not detectable in control glomeruli (**C**), whereas a CNF glomerulus shows moderate mesangial immunoreactivity (**D**). A weak immunoreactivity with antibodies detecting EDBFn in CNF in Bowman's capsules is shown by an arrow in Fig. 5E. **A–D** ×330, **E** ×130

Fig. 5A–H Location of laminin subunits. The immunoreactivity for α1 chain of laminin was found to be similar in all basement membranes in control (**A**) and CNF (**B**), kidneys whereas a stronger mesangial reactivity for α2 chain of laminin was noted in CNF (**D**) than in control (**C**) kidneys. A strong mesangial immunoreactivity in CNF kidneys (**F**) was also detected for β1 chain of laminin, in contrast to the moderate reactivity in controls (**E**). The immunoreactivity for β2 chain of laminin was seen as a continuous line in the GBM in both control (**G**) and CNF (**H**) kidneys. **A, B, G, H** ×130, **C–F** ×330

Discussion

CNF is clinically a very well characterised syndrome [16]. Despite the accumulation of the cases of CNF in Finland, this disease is met throughout the world and is one of the few syndromes with primary proteinuria starting in utero [30]. The pathogenetic mechanism leading to proteinuria in this disease is not yet known, although a faulty synthesis of some GBM component has been suspected. No defects in genes coding GBM components

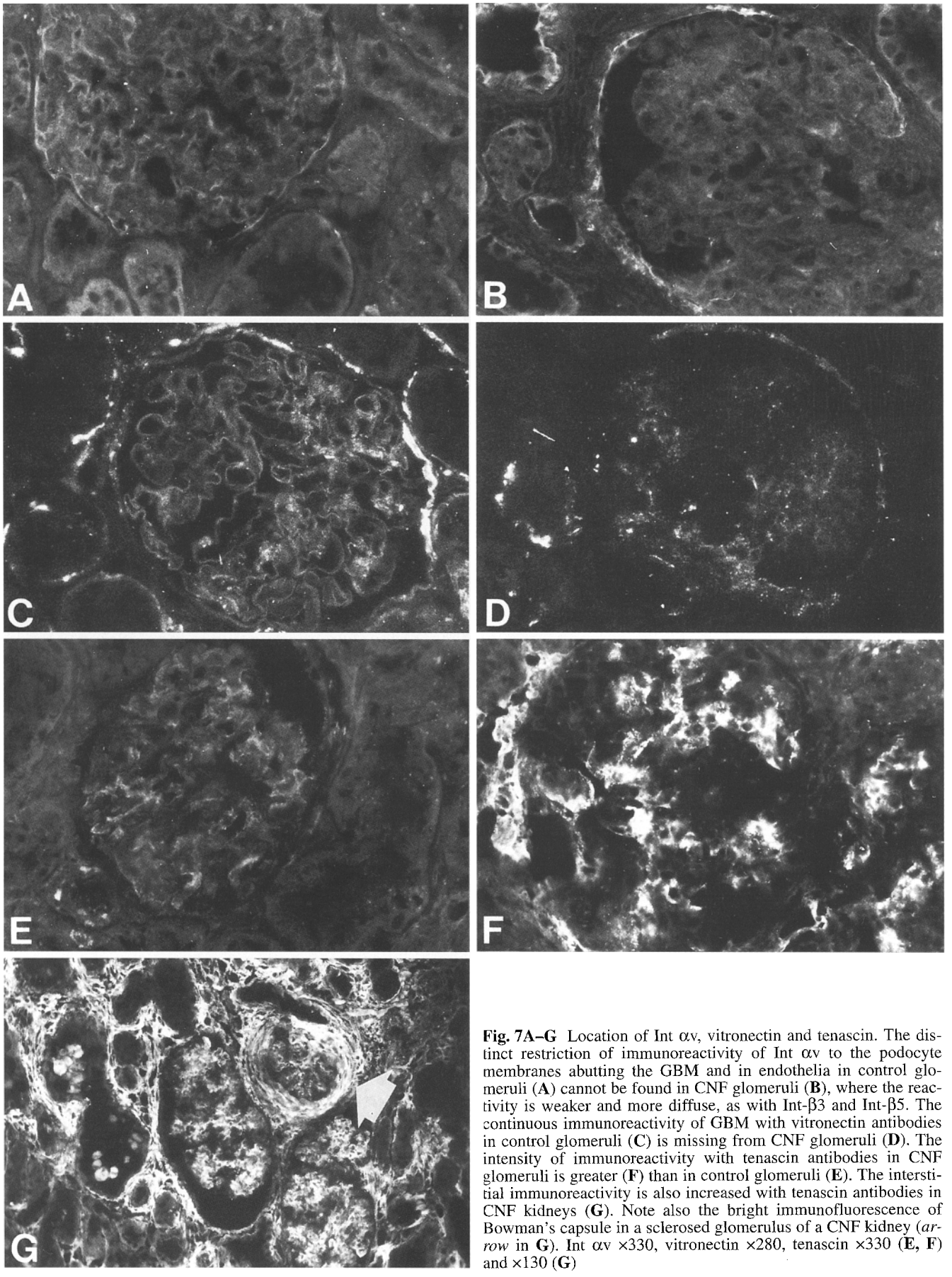

Fig. 7A–G Location of Int αv, vitronectin and tenascin. The distinct restriction of immunoreactivity of Int αv to the podocyte membranes abutting the GBM and in endothelia in control glomeruli (**A**) cannot be found in CNF glomeruli (**B**), where the reactivity is weaker and more diffuse, as with Int-β3 and Int-β5. The continuous immunoreactivity of GBM with vitronectin antibodies in control glomeruli (**C**) is missing from CNF glomeruli (**D**). The intensity of immunoreactivity with tenascin antibodies in CNF glomeruli is greater (**F**) than in control glomeruli (**E**). The interstitial immunoreactivity is also increased with tenascin antibodies in CNF kidneys (**G**). Note also the bright immunofluorescence of Bowman's capsule in a sclerosed glomerulus of a CNF kidney (*arrow* in **G**). Int αv ×330, vitronectin ×280, tenascin ×330 (**E, F**) and ×130 (**G**)

[20] or absence of GBM components in immunofluorescence staining have been found, although several abnormalities, mainly accumulation of ECM molecules, have been detected [20, 27]. In this study we examined the β1-subgroup of Ints, the most thoroughly studied receptors for ECM components and some of their ligands of the extracellular matrix in the CNF kidney sections with indirect immunofluorescence microscopy (IFL). In addition, we here describe for the first time the distribution pattern of αv Ints in normal and congenitally nephrotic kidneys.

Age-matched nephrectomised control kidneys were not available and thus could not be used as controls. The use of autopsy material as control tissue for nephrectomised kidneys is not satisfactory in our opinion. In addition, the expression pattern of β_1, β_3, and α_1-, α6 integrins in the most mature parts of fetal kidneys is similar to that in adult kidneys [22, 23]. Consequently, the use of adult nephrectomised kidneys as controls for CNF kidneys was considered suitable and acceptable.

The results revealed several differences in the intensity or location of the immunostaining for subunits of Int β1 and αv heterodimers and their ligands in CNF kidneys and controls. The mesangial reactivity for Int α1 and β1 subunits was more variable and an increased glomerular staining with Int α3 and α6 mAbs was found in CNF kidneys than in controls. In addition to these distinct findings, Int α2 subunit was either completely missing or found in significantly reduced amounts in CNF kidney glomeruli studied by IFL. The reactivity for Int-αv mAbs was more variable, fainter and also more granular in CNF samples than in control kidneys. In CNF kidneys the glomerular staining for Int β5 mAbs was more diffuse and weaker than in controls, whereas the reactivity in sclerotic Bowman's capsules was more intense in CNF kidneys than in controls. The immunoreactivity with Int β6 mAbs was comparable in control and CNF kidneys.

Three studies on the integrin expression in nephrotic kidneys have been performed to our knowledge [2, 4, 18]. First, Kanahara et al. [18] studied the distribution of fibronectin, vitronectin, fibronectin receptor and vitronectin receptor in IgA nephropathy with the indirect immunoperoxidase method: an expanded mesangial region accompanied higher fibronectin, fibronectin and vitronectin receptor distribution. The amount of these structures also increased significantly with the degree of histological damage. In the present study we could not find the classical fibronectin receptor (Int α5β1) in normal or nephrotic kidneys. This result has been confirmed with several mAbs against Int α5 subunit and with different detection methods (Virtanen et al, unpublished results). We also found the Int αv subunit to be present in smaller amounts and more granular in immunostaining pattern than controls. Consequently, the amount of any possible αvβ3 Int must also be decreased in CNF kidneys. Thus, at least the distribution of these integrin receptors in CNF kidneys differs distinctly from those of controls or IgA nephropathic kidneys.

Secondly, Baraldi et al. [4] described patchy and decreased staining of glomeruli with α3-Int mAbs in stage III mesangiocapillary glomerulonephritis. We found no such decrease in CNF kidneys. In contrast, the intensity of reactivity with Int α3 mAbs was increased in CNF kidney glomeruli. Of the known ligands for α3 Ints, at least laminin and cellular fibronectins were found to show an increased immunostaining pattern with indirect immunofluorescence microscopy in this study. The increase in both α3 and α6 Int immunostaining patterns may be a consequence of this increase of their ligands in ECM. Interestingly, Int α3 also binds nidogen: by using immunoblotting we found altered proportions of nidogen fragments in CNF GBM compared with controls [29]. If this is due to altered proteolysis of nidogen, this may lead to altered nidogen–α3-Int interaction in CNF kidneys, with a consequent change in α3 Int expression. Futhermore, the binding of fragments of fibronectin molecule to α5β1 Int increases the synthesis of matrix-degrading metalloproteases [43]. Whether other ECM protein fragments, such as the nidogen fragment, binding to Int α3β1 have effects on matrix degrading enzymes remains to be studied.

In a third study on Ints in nephrotic kidneys, Ahn et al. [2] found a glomerular increase in the immunoreactivity for Int α6 in lupus nephritis and IgA nephropathy. α6β1 Int is normally found only in the endothelium of kidney glomerulus, and the glomerular staining is weak [22, 31, 36]. Int α6 subunit is known to have at least two subtypes, with different cytoplasmic variants named α6A and α6B [8, 38], both being activation-dependent receptors for different isoforms of laminin [10]. Glomeruli have been reactive only with antibodies detecting both these isoforms, and not with isoform specific antibodies [15]. However, Ahn et al. reported that α6A was found in glomerular podocytes, whereas α6B was localised to tubular epithelium. As already mentioned, in CNF kidneys the staining pattern of α6-Int with MAb GoH3 recognising both isoforms was distinctly different from that in controls, being strong and reminiscent of the capillary loop in pattern. This agrees with the study by Ahn et al. [2] with nephrotic kidneys. Laminin-1, laminin-2 and laminin-5 belong to the known ligands for α6β1 Int [11], and we also found the expression of Ln β1 and α2 chains to be enhanced in CNF kidney glomeruli. Thus, the increase of Int-α6 subunit in CNF kidney glomeruli could reflect the increased demand for laminin binding of glomerular cells.

The interaction between Int α6 subunit with the E8 fragment of laminin is known to be essential for the epithelial polarisation in developing kidney tubules [37]. In CNF kidneys the overall structure is normal, and a major defect in epithelial polarisation seems unlikely. In contrast to Int α3β1 and α6β1, Ints α2β1 and α1β1 both bind to the cross-region of laminin [13, 17]. Thus, the altered expression pattern of Ints in CNF glomeruli may reflect an altered three-dimensional structure of glomerular basement membrane or mesangial matrix resulting in different epitope presentation of ECM molecules for cell adhesion by Int receptors.

The loss or decrease of Int α2 has not been described earlier in kidney diseases and is thus a novel finding. The known ligands in extracellular matrix for Int α2β1 are laminin-1, type I and IV collagens and tenascin. The amount of tenascin was either comparable to that in controls or increased in CNF glomeruli. Thus, the decrease of Int α2β1 cannot reflect the amount of tenascin in nephrotic glomeruli. Type I and IV collagens are also ligands for Int α2β1, but were not studied here. We have, however, described the location of type IV collagen in CNF kidneys earlier [28]: with polyclonal antibodies this ECM component was found to be somewhat unevenly distibuted in CNF GBMs. In conclusion, the decrease in Int α2β1 may result from a total absence of some of its ligands. Another possibility is that the binding site/sites of Int α2β1 in vivo in CNF kidney glomeruli is/are masked by some conformational change in the extracellular matrix.

We describe here the distribution of Int αv subunit and its partners. The location and immunostaining intensity of Int αv subunit and of Int β3 and β5 subunits were by and large similar, so that glomerular and tubular reactivities were weaker and more granular in pattern than in controls, whereas the immunostaining of Bowman's capsules was stronger, especially in sclerotic glomeruli. Vitronectin is possibly the most thoroughly studied ligand of Int αvβ3 and also a ligand for Int αvβ5. It is therefore not surprising that the immunostaining pattern given by anti-vitronectin was also weaker and more granular in CNF glomeruli and tubules than in controls. As a serum protein produced by liver with an M_r of 84 kDa [44], vitronectin is most probably readily lost into urine in these nephrotic patients. Thus, the decreased amount of vitronectin in CNF glomeruli could be secondary to the proteinuria. If the loss of αvβ3 and αvβ5 Ints in CNF kidneys is due to the loss of vitronectin, we might suggest that at least one of the functions of these integrins in normal glomerular podocytes is to mediate attachment or adhesion to the vitronectin molecules trapped in glomerular ECM from the blood circulation. A further potential partner for Int αv subunit is the Int β6 subunit, which has not yet been studied in human kidney. Breuss et al. [6] have described the distribution of Int β6 subunit in primate epithelial tissues and reported that high levels of Int β6 mRNA were found only in two types of epithelial cells: in kidney macula densa and in endometrial epithelium. In the present study, the Int β6 subunit was found at similar levels in normal and CNF kidneys in some tubules in a wide distribution. The function of Int β6 subunit in human kidneys remains to be studied.

The distribution of cellular fibronectins was also studied here. It was reported earlier [25] that they were differentially expressed in human fetal and adult kidneys: in adult kidneys only EDAFn can be observed in mesangium and endothelium, whereas in fetal kidneys these three forms of cFN have differences in their distribution, suggesting differential functions also. In CNF kidneys the glomerular reactivity with monoclonal antibodies detecting the EDAFn varied between and within glomeruli. An interstital increase and strong staining of Bowman's capsules of EDAFn were also found. Faint expression of EDBFn in glomerular mesangia and Bowman's capsules in CNF kidneys was also discovered. In addition, a faint reactivity with antibodies detecting oncFn in these locations was also found, as was a strong interstitial increase in tenascin reactivity in CNF kidneys. These kind of alterations in ECM composition have also been found in acute and chronic renal allograft rejection [12] and may thus represent nonspecific reaction to renal injury.

It has been suggested that structures resembling focal contacts exist between mesangial cells and GBM both in vivo and in cultured mesangial cells [24, 32]. This is thought to mean that any alterations in these contacts will alter the regulatory action of mesangial cells on glomerular haemodynamics [9]. We could not find any major losses or defects in the immunoreactivity for Int β1 or talin. Structures located in focal contacts include focal adhesion kinase [14] and urokinase-type plasminogen activator [33], and these are important for the signal-transducing mechanisms associated with integrin receptors and for degradation and remodelling of the extracellular. In this study, immunoreactivity for $pp125^{FAK}$ was not detectable in either control or CNF glomeruli. Thus, the Int β1-mediated adhesion of glomerular cells to extracellular matrix seems to be undisrupted in CNF kidneys, at least in kidneys removed from patients around 1 year old.

In conclusion, all the integrins and basement membrane components studied were present in CNF kidneys, albeit often in a distinctly altered manner as compared to control kidneys. Thus, a defect in any of these integrins seems a most unlikely cause of the proteinuria in CNF. However, this proteinuria is massive, and another human disease with such massive proteinuria persisting for 1 year is hard to find. Thus, the altered expression of integrins in this disease may be nonspecific and secondary to the long-lasting, massive proteinuria and the accumulation of several extracellular matrix components, as in several other glomerulopathies. Alterations in integrin expression have not been found in any other proteinuric disease in the same pattern as described here. At present, the typically disturbed integrin expression pattern in CNF may be thought specifically to reflect the disturbance of glomerular function caused by the primary defect in this disease, and a defect in some integrin-associated enzyme or signal-transducing pathway associated with integrins is not ruled out. Further studies are needed to examine these hypotheses.

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