The α_1–α_6 Subunits of Integrins Are Characteristically Expressed in Distinct Segments of Developing and Adult Human Nephron

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Abstract. We studied the distribution of the α_1–α_6 subunits of β_1 integrins in developing and adult human kidney using a panel of mAbs in indirect immunofluorescence microscopy.

Uninduced mesenchyme displayed a diffuse immunoreactivity for only the α_1 integrin subunit. At the S-shaped body stage of nephron development, several of the α subunits were characteristically expressed in distinct fetal nephron segments, and the pattern was retained also in the adult nephron. Thus, the α_1 subunit was characteristically expressed in mesangial and endothelial cells, the α_2 in glomerular endothelium and distal tubules, the α_3 in podocytes, Bowman's capsule, and distal tubules, and the α_6 subunit basally in all tubules, and only transiently in podocytes during development. Unlike the α_3 and α_6 subunits, the α_2 subunit displayed an overall cell surface distribution in distal tubules. It was also distinctly expressed in glomerular endothelia during glomerulogenesis. The β_4 subunit was expressed only in fetal collecting ducts, and hence the α_6 subunit seems to be complexed with the β_1 rather than β_4 subunit in human kidney. Of the two fibronectin receptor α subunits, α_4 and α_5, only the latter was expressed, confined to endothelia of developing and adult blood vessels, suggesting that these receptor complexes play a minor role during nephrogenesis.

The present results suggest that distinct integrins play a role during differentiation of specific nephron segments. They also indicate that $\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrin complexes may function as basement membrane receptors in podocytes and tubular epithelial cells.

THE interactions of cells with the extracellular matrix (ECM)[1] have raised considerable attention in the context of cell differentiation and tissue morphogenesis (Hay, 1983; Ekblom et al., 1986; Ekblom, 1989; Sanes, 1989). The expression of ECM proteins is under temporal and spatial control during development: for instance, distinct changes in the expression of fibronectins (Ekblom, 1981), interstitial collagens (Ekblom et al., 1981*a*) and basement membrane (BM) constituents (Ekblom et al., 1980; Ekblom, 1981; Ekblom et al., 1990) appear to accompany nephrogenesis. Such changes undoubtedly contribute to the mechanisms of orchestration of cell behavior during development.

Various ECM proteins appear to exert their influence by interacting with distinct cell surface receptors, called integrins (reviewed by Hynes, 1987; Ruoslahti and Giancotti, 1989). Until recently, in mammalian cells the integrin protein family has been divided into three subfamilies that each share a common β subunit. Recent findings, however, have revealed a more complex picture (Ruoslahti and Giancotti, 1989). Six different α subunits have thus far been shown to form complexes with the β_1 subunit (see Table I). Together, the various integrins enable cells to recognize a multitude of extracellular matrix proteins.

Thus far, knowledge of the functions of integrins has accumulated mainly on the basis of cell culture studies and little is known about their functions in tissues. Studies on both the avian CSAT antigen, corresponding to mammalian β_1 integrins (Hynes et al., 1989), as well as on mammalian integrins, have revealed that they may function in cell migration and neurite outgrowth (Bronner-Fraser, 1985, 1986; Krotoski et al., 1986; Hall et al., 1987), stabilization of tissue organization (Duband et al., 1986; Chen et al., 1986), transduction of differentiation signals (Menko and Boettiger, 1987; Adams and Watt, 1989; Hedin et al., 1989), and organization of the ECM (Giancotti and Ruoslahti, 1990).

Nephrogenesis (see Saxén, 1987; Bacallao and Fine, 1989) provides a good tissue model to study the functions of integrins. The primary vesicle is formed by induction from an apparently homogenous cell mass, the metanephric mesenchyme. It then differentiates into a mature nephron via the comma-shaped, S-shaped body, and capillary loop stages. These events present several cytodifferentiation models, and several modes of cell–matrix interactions for study.

The distribution of the β_1 integrin subunit in kidneys of various species has been considered in a few earlier studies (de Strooper et al., 1989; Fujimoto and Singer, 1988; Ker-

1. *Abbreviations used in this paper*: BM, basement membrane; ECM, extracellular matrix; GBM, glomerular basement membrane; PTA, *Psophocarpus tetragonolobus* agglutinin; TH, Tamm-Horsfall protein.

Table I. β_1 Integrin-related Complexes and Their Ligands

Receptor	Suggested ligands	References
$\alpha_1\beta_1$	Collagen IV (+I)	Kramer and Marks, 1989
$\alpha_2\beta_1$	Collagens, laminin	Wayner and Carter, 1987; Languino et al., 1989
	Cell–cell	Larjava et al., 1990
$\alpha_3\beta_1$	Laminin, fibronectin, and collagens I + VI	Wayner and Carter, 1987; Gehlsen et al., 1989
	Cell–cell	Larjava et al., 1990
$\alpha_4\beta_1$	Fibronectin	Wayner et al., 1989
	Lymphocyte homing	Holzmann et al., 1989
$\alpha_4\beta_p$	Lymphocyte homing	Holzmann and Weissman, 1989
$\alpha_5\beta_1$	Fibronectin	Pytela et al., 1985
$\alpha_6\beta_1$	Laminin	Sonnenberg et al., 1988
$\alpha_6\beta_4$	?	Hemler et al., 1989; Kajiji et al., 1989
$\alpha_7\beta_1$	Laminin	Kramer et al., 1989

jaschki et al., 1989). We have recently shown by immunohistochemistry that during nephrogenesis β_1 integrins become distinctly polarized both in glomerular endothelial cells and podocytes, as well as in proximal tubular epithelial cells (Korhonen et al., 1990). Furthermore, the basal organization of β_1 integrins appeared to take place concomitantly to the reorganization of talin, a cytoskeletal protein associated with the cytoplasmic aspect of the cell surface membrane.

Here we have further characterized the role of β_1 integrins in the formation and maintenance of tissue organization in human kidney by studying the distribution of the α_1–α_6 integrin subunits in developing and adult human kidney. The results suggest that distinct integrins play a segment-specific role during the maturation of the nephron. They also suggest that cells use different integrins as BM receptors.

Materials and Methods

Tissues

Adult human kidney samples (n = 20) were obtained from the clinically normal part of kidneys removed for renal cancer at the Jorvi Hospital (Espoo, Finland). The fetal kidneys (n = 4) were obtained from fetuses legally aborted at 14–20 wk of gestation, due to severe maternal or fetal complications, at the Department of Obstetrics and Gynecology (University Central Hospital, Helsinki). The tissues were immediately frozen in melting freon, cooled in liquid nitrogen, or directly in liquid nitrogen, and stored at −70°C until used.

Antibodies

The following mAbs against integrin subunits were used: the mAb 102DF5, recognizing the β_1 (Ylänne and Virtanen, 1989); the mAb S3-41, recognizing the β_4 (Kajiji et al., 1987); the mAb TS2/7, recognizing the α_1 (Hemler et al., 1984); the mAb CLB-10G11, recognizing the α_2 (Giltay et al., 1989); the mAb J143, recognizing the α_3 (Fradet et al., 1984; Hemler et al., 1987*a*); the mAb B-5G10, recognizing the α_4 (Hemler et al., 1987*b*); the mAb BIE5, recognizing the α_5 (Werb et al., 1989); and the mAb GoH3, recognizing the α_6 integrin subunit (Sonnenberg et al., 1987). These mAbs will be referred to in the text as anti-β_1 and -β_4, as well as anti-α_1 to -α_6, respectively. Anti-β_1, -α_5, and -α_6 mAbs were used as culture supernatants of the respective hybridomas, while anti-β_4, -α_1, -α_2, -α_3, and -α_4 were used as diluted ascites fluid. Anti-α_5 and -α_6 are rat, and the rest mouse mAbs.

In double-immunofluorescence labeling experiments FITC-coupled *Psophocarpus tetragonolobus* agglutinin (PTA; Sigma Chemical Co., St. Louis, MO) was used to visualize endothelia (Laitinen et al., 1990). Rabbit anti-collagen IV serum and rabbit anti-laminin serum (Liesi et al., 1983) were used to visualize BMs in double immunostaining experiments. Rabbit anti-Tamm-Horsfall protein (anti-TH) serum (Ekblom et al., 1981*b*), was used in double-labeling experiments to identify ascending limbs of Henle's loops and distal tubules.

Indirect Immunofluorescence Technique

Frozen sections (cut at 5 μm) were fixed in acetone, cooled to −20°C for 5 min. Then, they were incubated with the mAbs for 30 min, and subsequently with TRITC-coupled sheep anti-mouse IgG serum (Cappel, Organon Teknika Corp., West Chester, PA) or FITC-coupled goat anti-rat IgG serum (Cappel) for 30 min. In double-labeling experiments the sections were further incubated with polyclonal rabbit antiserum for 30 min, followed by FITC-coupled sheep anti-rabbit IgG serum (Cappel Laboratories) for another 30 min, or were exposed to the labeled lectins for 30 min. The fluorochrome-coupled second antibodies did not give any reaction when applied alone on the specimens. In immunostainings the sections were mounted in sodium veronal-glycerol buffer (1:1; pH 8.4). In lectin double-labeling experiments the specimens were embedded in Mowiol (Merck Ag., Darmstadt, FRG). A Leitz Aristoplan microscope, equipped with appropriate filters and phase-contrast optics was used to examine the specimens. In double exposures of double immunostainings, the same negative was exposed twice, first using FITC and then TRITC filters.

Results

α_1 Integrin Subunit Was Expressed in Undifferentiated Mesenchyme, and Mesangial and Endothelial Cells

In the fetal kidney, anti-α_1 (mAb TS2/7) reacted with undifferentiated mesenchyme. The induced, condensing mesenchyme lacked this reactivity, and primary vesicles were likewise negative. In S-shaped body structures, only the invading cells within the glomerular cleft, which are thought to consist of mesangial and endothelial cells (see Saxén, 1987), reacted (Fig. 1, *a* and *b*; double immunostaining with anti-laminin serum). Mesenchymally confined immunoreactivity was seen in the interstitium (Fig. 1, *a* and *c*). In capillary loop stage glomeruli, α_1 immunoreactivity was confined to the mesangial area (Fig. 1, *c* and *d*; double immunostaining with anti-laminin serum). This pattern continued throughout glomerulogenesis, and in the adult nephron, prominent α_1 immunoreactivity was seen in mesangial cells, while endothelial cells reacted more weakly (Fig. 1, *e* and *f*; double labeling with FITC-PTA). Anti-α_1 also reacted faintly with intertubular tissue, including the capillaries, as well as with walls and endothelia of arteries (not shown).

α_2 Integrin Subunit Characterized Glomerular Endothelial and Distal Tubular Epithelial Cells

In the fetal kidney, anti-α_2 (mAb CLB-10G11) reacted with the branching cortical, but not the medullary collecting ducts, and failed to react with undifferentiated cells, condensing mesenchyme, and primary vesicles. In early capillary loop stage glomeruli, immunoreactivity was detected in glomerular endothelial cells (Fig. 2, *a* and *b*; double immunostaining with anti-laminin serum).

At the S-shaped body stage, but more prominently in early capillary loop stage nephrons, anti-α_2 revealed an overall cell surface reactivity in the forming distal tubules (Fig. 2 *a*). In the subcortical parts of developing kidneys, α_2 immunoreactivity (Fig. 2 *c*) largely coincided with that of anti-TH serum (Fig. 2 *d*), used to reveal distal tubules. In adult tubules, anti-α_2 (Fig. 2 *e*) revealed an overall cell sur-

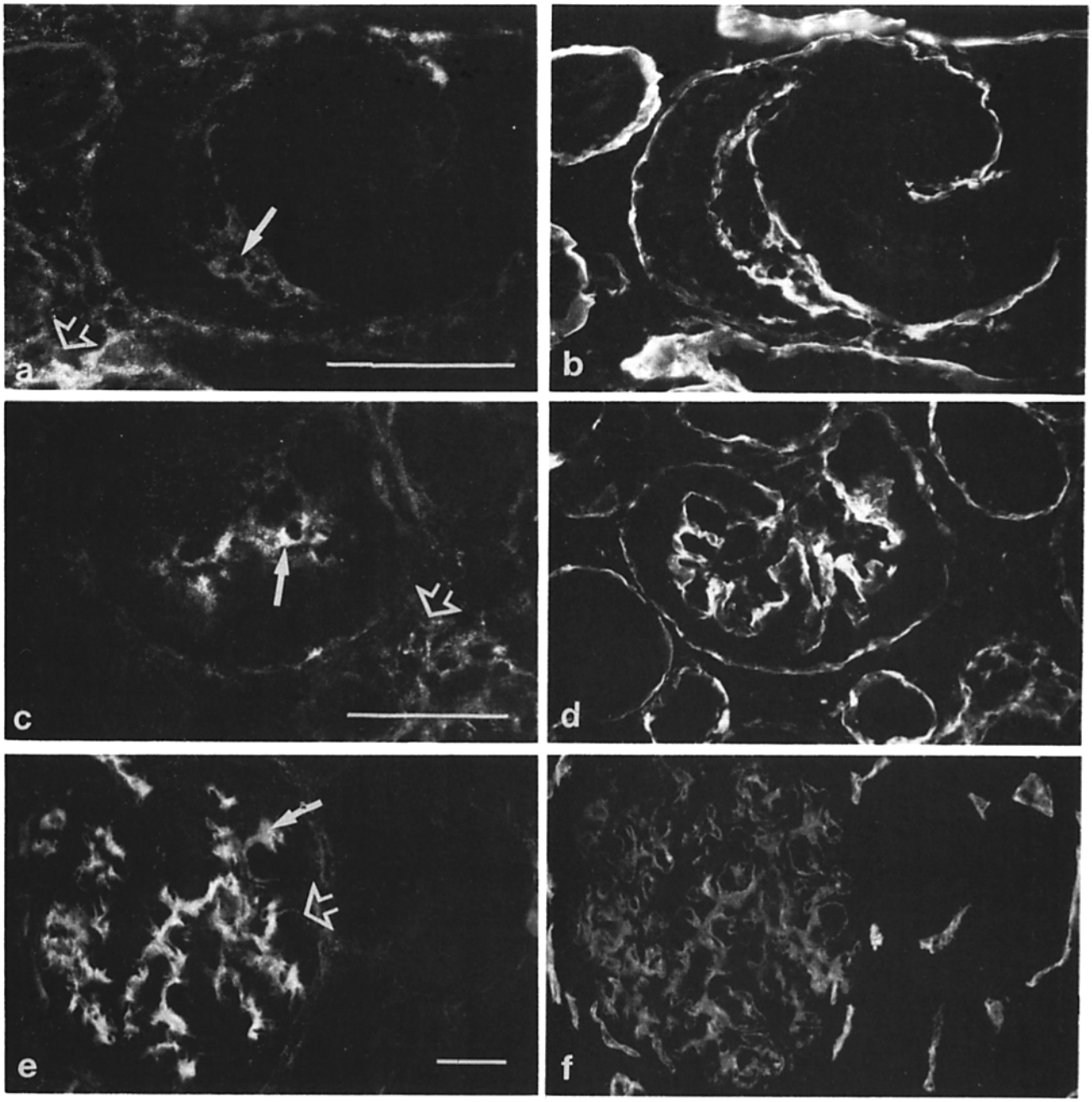

Figure 1. Localization of the α_1 subunit in 16-wk fetal (*a–d*) and adult (*e* and *f*) kidneys. (*a* and *b*) Anti-α_1 (*a*) reacts with invading cells (*arrow*) within the glomerular crevice of the S-shaped body. Double immunostaining with anti-laminin serum (*b*) to reveal BMs. (*c* and *d*) In capillary loop stage glomeruli, anti-α_1 (*c*) reveals immunoreactivity in the mesangial area (*arrow*). (*d*) Double immunostaining with anti-laminin serum. (*e* and *f*) In adult glomeruli, anti-α_1 (*e*) reacts prominently with mesangial areas (*arrow*), and more weakly with endothelia (*open arrow*). Double labeling using FITC-PTA (*f*) to reveal glomerular and intertubular endothelia. Note the diffuse mesenchymal immunoreactivity in the fetal sections (*a* and *c*, *open arrows*), and the weak intertubular immunoreactivity in the adult (*e*). Bars, 50 μm.

face immunoreactivity in certain tubular segments, principally coinciding with that obtained with anti-TH (Fig. 2 *f*). This, together with morphological criteria, suggests that in the adult the α_2 integrin subunit is expressed in distal tubules and collecting ducts.

In adult glomeruli, anti-α_2 (Fig. 2 *g*) reacted very weakly, codistributing with FITC-PTA binding (Fig. 2 *h*), which was used to reveal endothelia. However, immunoelectron microscopy is needed in order to ascertain whether the α_2 subunit is confined to endothelial cells, which was more prominently seen in fetal samples. Furthermore, endothelia of arteries, but not of intertubular capillaries, reacted with anti-α_2 in adult kidney (not shown).

α_3 Integrin Subunit Was Expressed in Podocytes, Bowman's Capsule, and the Distal Tubule

Anti-α_3 (mAb J143) revealed no reactivity in undifferentiated or condensed mesenchyme, nor in fetal collecting ducts or primary vesicles. At the early S-shaped body stage, however, the presumptive glomerular podocytes, as well as the

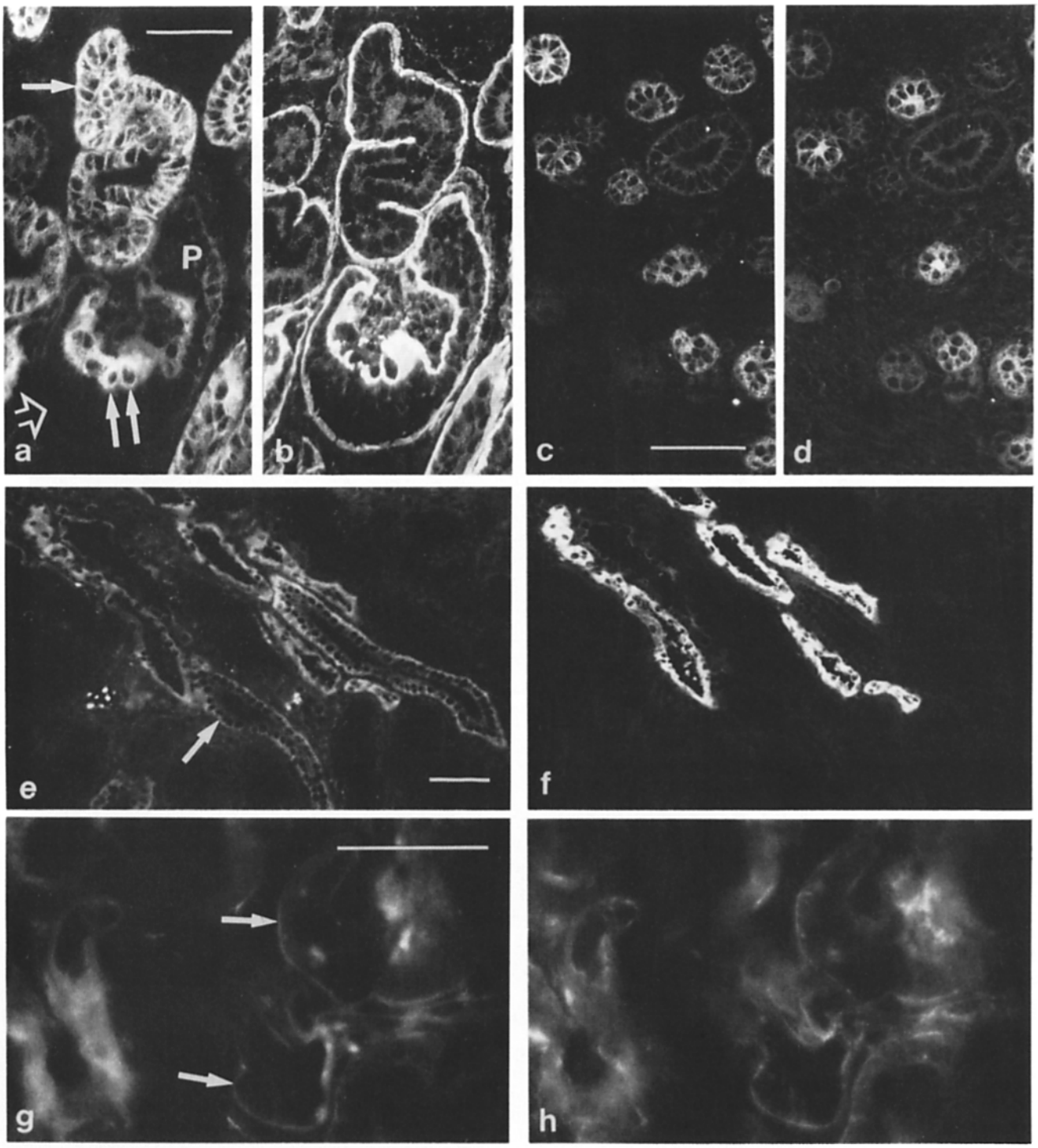

Figure 2. Localization of the α_2 subunit in 16-wk fetal (*a–d*) and adult (*e–h*) kidneys. (*a* and *b*) In an early capillary loop stage nephron, anti-α_2 (*a*) reacts with glomerular endothelia (*double arrow*) and the cells of the distal tubule anlage (*arrow*), whereas the cells of the developing proximal tubule (*P*) and future Bowman's capsule (*open arrow*) are negative. (*b*) Double immunostaining with anti-laminin serum, revealing BMs. (*c–f*) Anti-α_2 reacts with distal tubules in the medullary region of a 16-wk fetal kidney (*c*) and of adult kidney cortex (*e*). The larger tubules that react in *e* (*arrow*) are collecting ducts. Double immunostainings with anti-TH (*d* and *f*) to reveal distal tubules. (*g* and *h*) In adult glomeruli, anti-α_2 (*g*, *arrows*) appears to colocalize with FITC-PTA (*h*) reactivity, used to reveal endothelia. Bars: (*a–e*) 50 μm; (*g*) 25 μm.

cells of the future Bowman's capsule, displayed cell membrane-confined anti-α_3 immunoreactivity (Fig. 3 *a*). In more mature S-shaped bodies, the reactivity was stronger and somewhat polarized along the glomerular basement membrane (GBM; Fig. 3, *b* and *c*; double immunostaining with anti-laminin serum). Polarization was even more marked in capillary loop stage glomeruli (Fig. 3 *d*; Fig. 3 *e* shows the corresponding phase-contrast view). In the adult kidney,

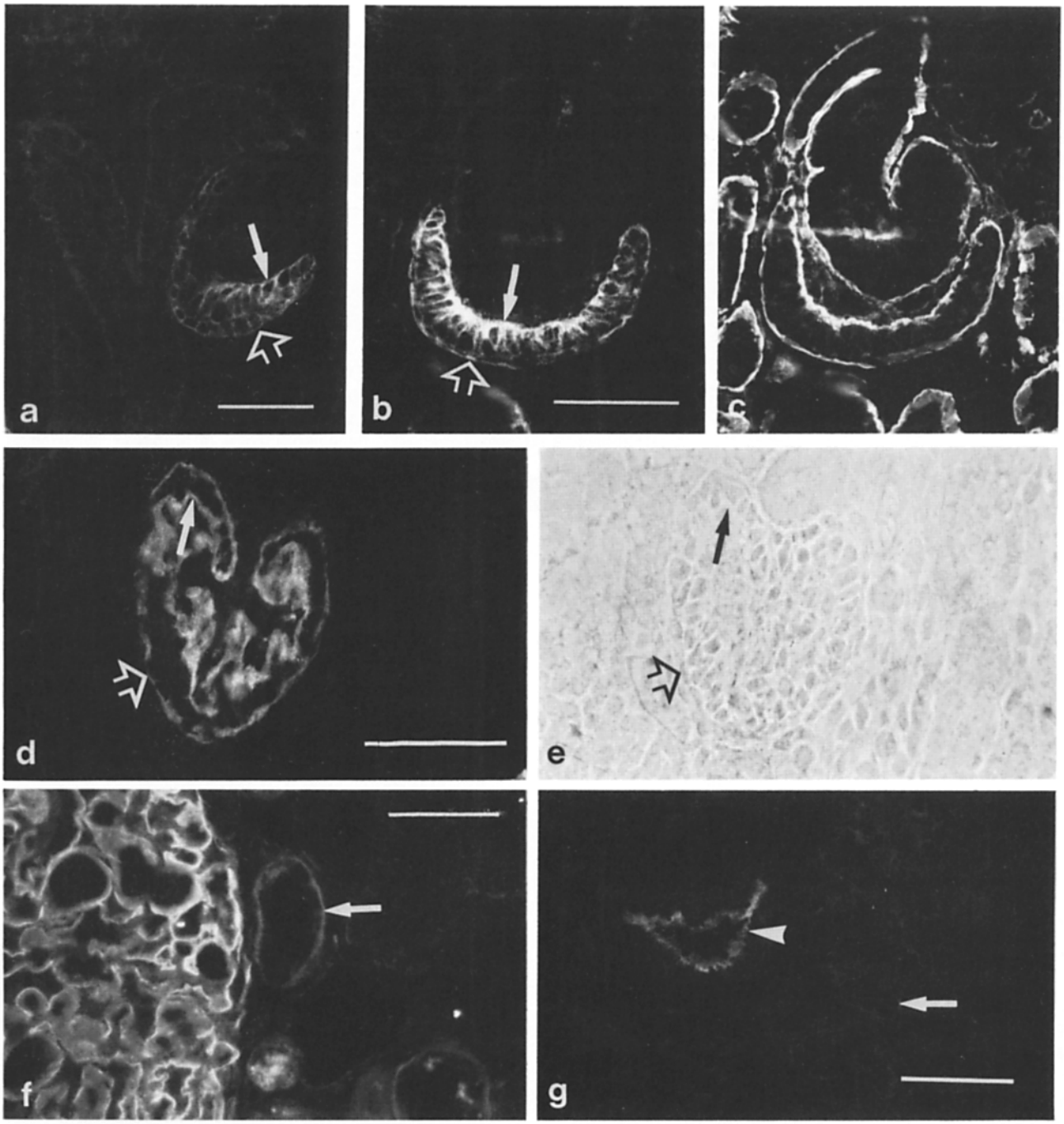

Figure 3. Localization of the α_3 subunit in 16-wk fetal (*a–e*) and adult (*f*), and of the α_5 subunit in 16-wk fetal (*g*) kidneys. (*a*) Nonpolarized anti-α_3 immunoreactivity is seen in presumptive podocytes (*arrow*) and Bowman's capsule cells (*open arrow*) in the early S-shaped body. (*b* and *c*) In an S-shaped body, anti-α_3 (*b*) reveals a beginning polarization of the α_3 subunit along the GBM in podocytes (*arrow*) as well as along the capsular BM in future Bowman's capsule cells (*open arrow*). Proximal and distal tubule anlagen are negative. Double immunostaining with anti-laminin serum (*c*) to reveal BMs. (*d* and *e*) In a capillary loop stage glomerulus, anti-α_3 reacts with podocytes (*d* and *e*, *arrows*) and cells of Bowman's capsule (*d* and *e*, *open arrows*). The corresponding phase-contrast view (*e*) reveals that the reactivity is confined to those parts of the podocyte cell membranes that abut the GBM (*arrows*). (*f*) In adult kidneys anti-α_3 reacts basally with distal tubules (*arrow*), in addition to reacting brightly along GBMs. (*g*) Anti-α_5 reacts with endothelia of larger blood vessels (*arrowhead*), and weakly with those of glomerular capillaries (*arrow*) in 16-wk fetal kidney. Bars, 50 μm.

anti-α_3 (Fig. 3 *f*) reacted basally with distal tubular epithelial cells, and distinctly with glomeruli, including Bowman's capsule. Corresponding tubular reactivity was not detected in 16-wk fetal distal tubules. Anti-α_3 reacted also with the walls of adult arteries (not shown). To study closer the nature of the anti-α_3 immunoreactivity in adult glomeruli, several double-labeling experiments were done. Anti-α_3 revealed single prominent lines of immunoreactivity, that surrounded the GBM loops revealed with anti-collagen IV serum (Fig. 4), or anti-laminin serum (not shown), or endothelial capil-

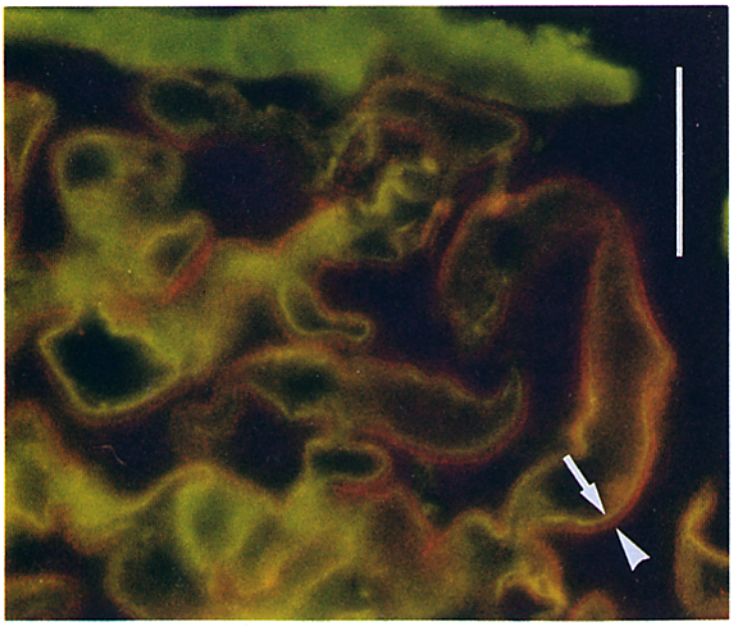

Figure 4. Double immunostaining of an adult glomerulus with anti-α_3 (red immunofluorescence) and anti-collagen IV serum (green immunofluorescence). The lines of anti-α_3 immunoreactivity (*arrowhead*) surround the GBM loops revealed by anti-collagen IV serum (*arrow*), suggesting that the α_3 subunit is expressed by podocytes. Bar, 25 μm.

lary loops revealed with FITC-PTA (not shown). Thus, the α_3 subunit appears to be confined to the podocyte membranes abutting the GBM.

The Fibronectin Receptors, α_4 and α_5 Integrins, Were Almost Completely Lacking in Kidney Tissue

Anti-α_4 (mAb B-5G10) failed to react with fetal and adult kidney tissue (not shown). Anti-α_5 (mAb BIE5) reacted weakly with endothelia of arteries and of glomerular and intertubular capillaries both in developing (Fig. 3 *g*) and adult (not shown) kidneys.

α_6 Integrin Subunit Was Basally Polarized in All Tubular Epithelial Cells and Transiently Expressed in Podocytes

Anti-α_6 (mAb GoH3) revealed a prominent immunoreactivity along the BMs of the branching collecting duct, but none was seen in uninduced mesenchyme. In primary vesicles, anti-α_6 (Fig. 5 *a*) revealed a line of basally polarized immunoreactivity, following the BM revealed with anti-laminin serum (Fig. 5 *b*). Furthermore, overall cell surface immunoreactivity for the α_6 subunit was detected in the cells in the glomerular pole of the vesicles (Fig. 5 *a*). However, no immunoreactivity for laminin was detected in this location. In S-shaped bodies, anti-α_6 reacted only weakly along the BM of Bowman's capsule, but revealed distinct immunoreactivity along the BMs of the developing proximal and distal tubules (Fig. 5 *c*).

In comma-shaped bodies, the future podocytes displayed a faint overall cell surface immunoreactivity for the α_6 subunit (not shown). With further development, a somewhat polarized reactivity was detected along the GBM (Fig. 5 *c*), possibly localizing both to endothelial cells and podocytes. At the capillary loop stage, anti-α_6 (Fig. 5 *d*) reacted weakly along the GBM, revealed by anti-laminin immunostaining (Fig. 5 *e*), and very weakly along the BM of Bowman's capsule. In all adult tubules, a distinct basally confined anti-α_6 reactivity was detected (Fig. 5 *f*). Furthermore, anti-α_6 reacted weakly with endothelia of arteries and of intertubular and glomerular capillaries (Fig. 5 *f*).

As the α_6 integrin subunit has been reported to be complexed with the β_4 subunit in some tissues (Hemler et al., 1989; Kajiji et al., 1989), we also studied the distribution of the β_4 subunit by using the mAb S3-41. Anti-β_4 reacted only with the collecting duct in fetal samples (Fig. 5 *g*), but revealed no immunoreactivity in adult kidneys. It also reacted with endothelia of larger blood vessels. These results suggest that the α_6 subunit is mainly complexed with the β_1 instead of the β_4 subunit in the human kidney.

Discussion

Knowledge of the tissue distribution of the individual β_1 integrins by use of α subunit-specific mAbs, and inferences on their functions in vivo are still fragmentary. In this study we have characterized the distribution of the α subunits of the β_1 integrin family in kidney, and show that during development their expression characteristically emerges in distinct nephron segments.

In previous studies, the localization of the α subunits of β_1 integrins in a given tissue has usually been taken to imply the presence of $\alpha\beta$ heterodimers. Provided that the possibility of α subunits complexing with more than one type of β subunit (Hemler et al., 1989; Holzmann and Weissman, 1989; Ruoslahti and Giancotti, 1989) is taken into account, we feel that such an assumption is justified. The inference that heterodimers, and not single subunits, are indeed being detected is supported by observations that uncomplexed α or β subunits do not reach the cell surface but are degraded in the cytoplasm (Springer et al., 1987; Heino et al., 1989; Rosa and McEver, 1989).

The Fibronectin Receptors, $\alpha_4\beta_1$ and $\alpha_5\beta_1$ Integrin Complexes, Play a Minor Role in Kidney Morphogenesis

Fibronectin has been implicated in several aspects of tissue

morphogenesis (Boucaut et al., 1984*a*, *b*; Ruoslahti, 1988; Rogers et al., 1989), and the fibronectin receptor $\alpha_5\beta_1$ complex has been widely studied as a "prototype" of integrins. Fibronectin is present in the basement membranes of the developing nephron, but disappears during development, although it is present throughout intertubular tissue in adult kidney (Vartio et al., 1987). In our study, however, the $\alpha_5\beta_1$ integrin was detected only in endothelia, and the $\alpha_4\beta_1$ complex, which has been suggested to bind the alternative cell-binding (CS-1) segment of fibronectin (Wayner et al., 1989), was not detected at all. Nor did the $\alpha_3\beta_1$ integrin colocalize with fibronectin. Thus, none of the fibronectin-binding integrins seemed to consistently codistribute with their ligand in kidney, although such an association has been suggested for some other developing tissues (Chen et al., 1986; Duband et al., 1986).

The $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_1$ Integrin Complexes Are Involved in Glomerulogenesis

Here we found that the $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_1$ integrins are expressed in characteristic glomerular cell types during nephrogenesis. This, and the transient expression of the $\alpha_6\beta_1$ integrin in podocytes during the early stages of glomerulogenesis, suggest that these complexes may play a part in nephrogenesis. In our earlier work, using mAbs to the β_1 subunit, we found pairs of distinct lines of immunoreactivity, one on each side of the GBM in the adult kidney (Korhonen et al., 1990). The present results suggest that the outermost lines may correspond to the $\alpha_3\beta_1$ complexes of podocytes. However, immunoelectron microscopy is needed to confirm its localization to podocytes. During development, the $\alpha_2\beta_1$ complex appeared to be the dominant endothelial integrin, while in the adult, no prevalent immunoreactivity for the various α subunits could be distinguished in endothelia. At the light microscopic level, all the anti-α_1, -α_2, -α_3, -α_5, and -α_6 integrin subunit mAbs appeared to reveal weak immunoreactivity in adult glomerular endothelia.

The $\alpha_6\beta_1$ Integrin Is Seen in All Tubules, and the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ Complexes Are Expressed in the Distal Tubule

The $\alpha_2\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_1$ integrin complexes were also detected at specific locations in kidney tubules. The strict basal confinement of the $\alpha_3\beta_1$ and $\alpha_6\beta_1$ complexes is most probably due to receptor–ligand interaction. It is in some contrast to the usual basolateral distribution of many integral membrane proteins of polarized epithelial cells (Gumbiner and Louvard, 1985). Interestingly, Na/K-ATPase is excluded from regions where the plasma membrane of tubular epithelial cells and the BM are opposed (Kashgarian et al., 1985; Morrow et al., 1989). Thus, the BM-associated $\alpha_6\beta_1$ integrin complexes may mark a microdomain where the Na/K-ATPase is absent.

In contrast to the polarized expression of the $\alpha_3\beta_1$ and $\alpha_6\beta_1$ integrins, the α_2 subunit showed an overall cell surface distribution in the distal tubule and collecting ducts. Earlier, we have reported the overall cell surface distribution of the β_1 subunit in distal tubules (Korhonen et al., 1990). According to a recent report, the $\alpha_2\beta_1$ complex may function in cell–cell interactions in cultured keratinocytes (Larjava et al., 1990); this activity would also explain its distribution in the distal tubule.

The $\alpha_3\beta_1$ and $\alpha_6\beta_1$ Integrin Complexes May Function as BM Receptors

The strict basal confinement of the $\alpha_3\beta_1$ and $\alpha_6\beta_1$ complexes suggests that these two different integrins may be used as BM receptors in the human kidney. The need for different receptors may arise from differences in BM structure in various locations. Alternatively, various integrins could bind to similar BM structures, but mediate different functions such as different cytoskeletal organization in the cells. Indeed, heterogeneity of BM composition in kidneys has been reported (Horikoshi et al., 1988; Desjardins and Bendayan, 1989; Abrahamson et al., 1989; Ekblom et al., 1990; Hunter et al., 1989).

A variety of receptors with affinity for laminin have been described, and the laminin molecule has several domains that may interact with receptors (see Beck et al., 1990). Possibly several laminin receptors are functional during nephrogenesis. In addition to integrins, the 67-kD laminin receptor has been studied in this context (Laurie et al., 1989), but different nephron segments were not identified. The carboxy-terminal part of the laminin A chain has been suggested to play a role in the morphogenesis of the tubular epithelium of the kidney, and the expression of this chain during development coincides with the onset of tubular morphogenesis (Klein et al., 1988; Ekblom, 1989; Ekblom et al., 1990). The carboxy-terminal end of the laminin molecule has been reported to be preferentially directed towards the epithelial cell surface in some BMs, including the proximal tubule (Schittny et al., 1988; Abrahamson et al., 1989), and furthermore, the $\alpha_6\beta_1$ integrin was recently shown to bind to the E8 fragment of laminin (Aumailley et al., 1990). In this respect it is of interest that the $\alpha_6\beta_1$ integrin is coexpressed with laminin A chain by tubular epithelial cells, and that the transient expression of the α_6 subunit by podocytes coincides with that of laminin A chain (this study; Ekblom et al., 1990). The $\alpha_6\beta_1$ complex is thus a good candidate for mediating the tubulogenic activity of laminin.

It is possible that the expression of various integrin receptors reflects the lines of differentiation of the cells. Recently, Languino et al. (1989) suggested that cell type–specific factors can modulate the ligand specificity of the $\alpha_2\beta_1$ complex in endothelial cells to include laminin. It would be interesting to know whether this receptor plays a role in mediating the putative angiogenic influence of laminin (Grant et al., 1989) during the morphogenesis of glomerular capillaries.

During tissue morphogenesis, the adhesive interactions of cells with each other and with the ECM are thought to play a crucial role (Hay, 1983; Ekblom et al., 1986). During nephrogenic differentiation the various nephron segments with their distinct cell types and properties arise from an apparently homogenous mesenchymal cell mass. Several classes of molecules have been studied in this context: ECM proteins such as fibronectin (Ekblom, 1981), tenascin (Aufderheide et al., 1987), collagens (Ekblom et al., 1981*a*), and laminin (Ekblom et al., 1980; Klein et al., 1988; Laurie et al., 1989; Ekblom et al., 1990), the ganglioside GD3 (Sariola et al., 1988), as well as some cell surface receptors such as uvomorulin (Vestweber et al., 1985), syndecan (Vainio et al.,

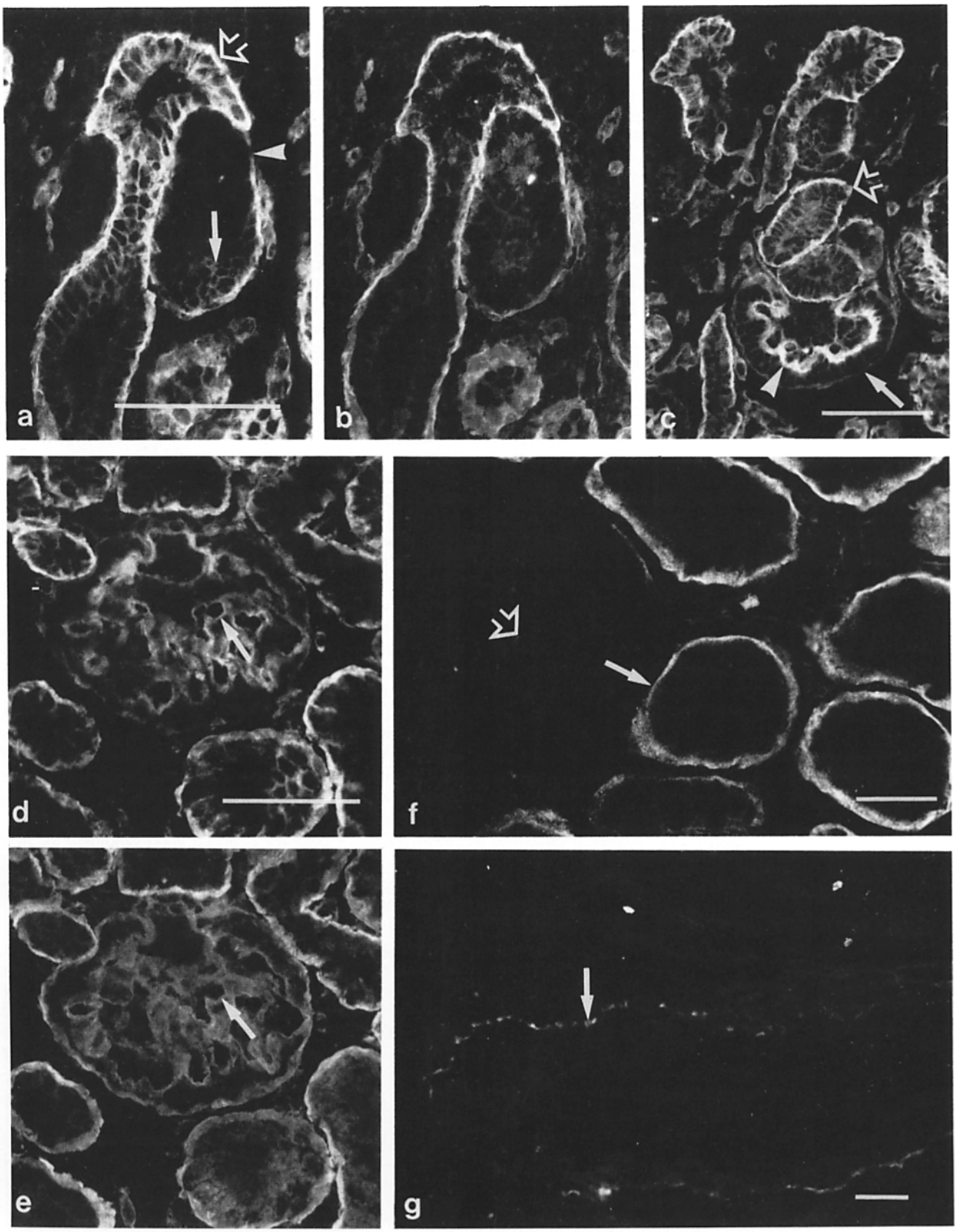

Figure 5. (*a* and *b*) Anti-α_6 reacts with the cells of the collecting duct (*open arrow*), and with the primary vesicle in a basally polarized manner (*arrowhead*) in 16-wk fetal kidney. It also reveals cell surface immunoreactivity in the distal pole of the vesicle, where the glomerular invagination forms during further development (*arrow*). Double immunostaining with anti-laminin serum (*b*) reveals basement membranes, and also that laminin does not colocalize with α_6 immunoreactivity in the cells of the distal pole of the vesicle. (*c*) In an early

1989), N- and P-cadherins (Takeichi, 1988), and the 67-kD laminin receptor (Laurie et al., 1989). The precise coordination of these interactions is instrumental in the morphogenesis of the kidney. The β_1 integrins provide an example of molecules involved in cell–ECM interactions displaying segment-specific expression. They may therefore play an important role in guiding the differentiation of distinct nephron segments.

The authors are indebted to Drs. Caroline Damsky (Department of Anatomy and Stomatology, University of California, San Francisco, CA), Martin Hemler (Dana-Farber Cancer Institute, Boston, MA), Päivi Liesi (Department of Biotechnology, University of Helsinki, Finland), Aaro Miettinen (Department of Bacteriology and Immunology, University of Helsinki, Finland), Jan van Mourik (Department of Immuno-Hematology, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), Lloyd J. Old (Ludvig Institute for Cancer Research, New York), Vito Quaranta (Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA), Leila Risteli (Department of Biochemistry, University of Oulu, Finland), and Arnoud Sonnenberg (Department of Immuno-Hematology, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) for the kind gift of antibodies used in the study. We would like to thank Dr. Peter Ekblom (Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tübingen, Federal Republic of Germany) for helpful discussions. The skillful technical assistance of Ms. Maija-Leena Johansson, Ms. Pipsa Kaipainen, Mr. Reijo Karppinen, Ms. Marja-Leena Piironen, and Ms. Hanna Wennäkoski is acknowledged.

This study was supported by a research contract with the Academy of Finland, and grants from the Finnish Cancer Research Fund, the Sigrid Juselius Foundation, the Paulo Foundation, the Emil Aaltonen Foundation and the Science Foundation of Farmos.

Received for publication 21 February 1990 and in revised form 23 May 1990.

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capillary loop stage glomerulus of 16-wk fetal kidney, anti-α_6 reacts prominently along tubular BMs (*open arrow*) but weakly along Bowman's capsule BM (*arrow*). Distinct immunoreactivity can also be seen along the GBM (*arrowhead*), possibly localizing both to podocytes and endothelia. (*d* and *e*) In a more developed capillary loop stage glomerulus, anti-α_6 (*d*) reacts less prominently along the GBM, possibly with endothelia (*d*, *arrow*), as the GBMs (*e*, *arrow*), revealed by double immunostaining with anti-laminin serum, appear to surround the anti-α_6 immunoreactivity. (*f*) In the adult kidney, prominent basally confined immunoreactivity for α_6 is seen in tubules (*arrow*), while glomerular (*open arrow*) capillaries react weakly. Weak reactivity, possibly representing capillaries, is also seen in intertubular tissue. (*e*) Anti-β_4 reacts basally with the collecting duct (*arrow*) in the 16-wk fetal kidney. Bars, 50 μm.

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