Recognition of the Laminin E8 Cell-binding Site by an Integrin Possessing the α_6 Subunit Is Essential for Epithelial Polarization In Developing Kidney Tubules

Lydia Sorokin,* Arnoud Sonnenberg,‡ Monique Aumailley,§ Rupert Timpl,§ and Peter Ekblom*

*Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, D-7400 Tübingen, Federal Republic of Germany; ‡Service of the Netherlands Red Cross Central Laboratory of Bloodtransfusion, Department of Immunohaematology, Amsterdam, The Netherlands; and §Max-Planck-Institut für Biochemie, D-8033 Martinsried, Federal Republic of Germany

Abstract. It has been previously shown that A-chain and domain(E8)-specific antibodies to laminin that inhibit cell adhesion also interfere with the establishment of epithelial cell polarity during kidney tubule development (Klein, G., M. Langegger, R. Timpl, and P. Ekblom. 1988. *Cell.* 55:331–341). A monoclonal antibody specific for the integrin α_6 subunit, which selectively blocks cell binding to E8, was used to study the receptors involved. Immunofluorescence staining of embryonic kidneys and of organ cultures of metanephric mesenchyme demonstrated coappearance of the integrin α_6 subunit and the laminin A-chain in regions where nonpolarized mesenchymal cells convert into polarized epithelial cells. Both epitopes showed marked colocalization in basal areas of tubules, while an exclusive immunostaining for α_6 was observed in lateral and apical cell surfaces of the tubular epithelial cells. Organ culture studies demonstrated a consistent inhibition of kidney epithelium development by antibodies against the α_6 subunit. The data suggest that the recognition of E8 cell-binding site of laminin by a specific integrin is crucial for the formation of kidney tubule epithelium from undifferentiated mesenchymal stem cells. In some other cell types (endothelium, some ureter cells) an exclusive expression of α_6 with no apparent colocalization of laminin A-chain in the corresponding basement membrane was seen. Thus, in these cells, integrins possessing the α_6 subunit may bind to laminin isoforms that differ from those synthesized by developing tubules.

POLARIZED epithelial cells play fundamental roles in the function of several mammalian tissues (Simons and Fuller, 1985; Rodriguez-Boulan and Nelson, 1989). Recent studies on kidney development have suggested that laminin, an extracellular glycoprotein found primarily in basement membranes, is important for the development of epithelial cell polarity (Klein et al., 1988). A well-characterized laminin isoform contains one 400-kD A-chain and two different 200-kD B-chains (Timpl, 1989). In the developing kidney, A-chain expression is restricted to epithelial cells, whereas the B-chains are also found in endothelium and in nonpolar mesenchymal cells. In organ cultures of embryonic kidney, antisera reacting with the E3 and E8 fragments of laminin inhibited development of mesenchyme into epithelium (Klein et al., 1988). These laminin fragments are located on the carboxy-terminal portion of the molecule and are composed either entirely (E3) or largely (E8) of A-chain sequences (Deutzmann et al., 1988), suggesting the involvement of carboxy-terminal portions of the A-chain of laminin in binding to epithelial cells.

In earlier studies laminin fragment E8 or related structures were shown to promote cell adhesion and spreading, as well as neurite outgrowth in vitro (Edgar et al., 1984; Au-

mailley et al., 1987; Goodman et al., 1987; Dillner et al., 1988). As in kidney development, these biological effects of laminin could be inhibited by antibodies to both fragments E8 and E3, the latter apparently by steric hindrance (Edgar et al., 1984). Fragment E8 was also shown to competitively inhibit laminin binding to cells (Nurcombe et al., 1989) indicating that it possesses a major cell-binding site. However, cellular recognition of laminin does not only involve the E8 fragment but is a more complex process also involving a latent RGD-dependent cell-binding site on fragment P1 (Nurcombe et al., 1989; Aumailley et al., 1990a), an unrelated P1 site responsible for mitogenic activity (Panayotou et al., 1989), and a possibly minor site on fragment E3 (Sonnenberg et al., 1990a). Further cell-binding sites have also been suggested from studies with synthetic peptides (Graf et al., 1987; Tashiro et al., 1989).

The complexity of the cellular recognition of laminin is also reflected by the increasing number of laminin receptors that have been identified (Lesot et al., 1983; Gehlsen et al., 1988; Sonnenberg et al., 1988a,b; Kleinman et al., 1988). Most of the receptors belong to the integrin family of plasma membrane–spanning proteins which consist of α and β subunits (Hynes, 1987; Ruoslahti and Pierschbacher, 1987;

Buck and Horwitz, 1987; Hemler et al., 1987). Laminin receptors from the β_1 subfamily, which differ in their α subunits, include $\alpha_1\beta_1$ or related structures (Ignatius and Reichardt, 1988; Turner et al., 1989; Forsberg et al., 1990; Rossino et al., 1990), $\alpha_2\beta_1$ (Languino et al., 1989; Elices and Hemler, 1989), $\alpha_3\beta_1$ (Gehlsen et al., 1988, 1989), $\alpha_6\beta_1$ (Sonnenberg et al., 1988b; Aumailley et al., 1990b), and some with unidentified α subunits (Douville et al., 1988; Kramer et al., 1989; Kajiji et al., 1989). Most of these integrins are promiscuous receptors because they also bind other matrix proteins apart from laminin (Tomaselli et al., 1988; Elices and Hemler, 1989; Hynes et al., 1989). However, $\alpha_6\beta_1$ appears to be specific for laminin and was recently shown to be the principal receptor for the E8 cell-binding site (Sonnenberg et al., 1990a; Aumailley et al., 1990b). Other parts of the laminin molecule, such as the P1 or E3 fragments, are also recognized by β_1 or β_3 integrins but do not include $\alpha_6\beta_1$ (Sonnenberg et al., 1990a,b). Further laminin receptors may include a single chain 67-70-kD protein (von der Mark and Kühl, 1985) recognizing a synthetic sequence of fragment P1 (Graf et al., 1987) and proteoglycans recognizing fragment E3 (Sonnenberg et al., 1990b).

Our previous observation of inhibition of kidney tubule development by domain-specific anti–laminin antibodies was interpreted as evidence for interference with cell binding to E8 structures (Klein et al., 1988). In agreement with this possibility is a concomitant strong expression of the laminin A-chain mRNA and the corresponding polypeptide in regions where epithelial cell polarization begins (Ekblom et al., 1990). Other biological activities attributed to the E8 and/or E3 domains include heparin binding (Ott et al., 1982) and self assembly of laminin (Charonis et al., 1986), raising the possibility that inhibition of kidney tubule development by antibodies to E8 or E3 may have been caused indirectly by a failure of proper basement membrane assembly. To distinguish between these possibilities we have now taken advantage of a monoclonal antibody (GoH3) specific for the α_6 integrin subunit which selectively blocks cell adhesion to E8 (Sonnenberg et al., 1986, 1987, 1988a,b, 1990a; Aumailley et al., 1990b).

Materials and Methods

Tissues and Organ Culture

Developing embryonic kidneys were dissected from 11-18 d-old hybrid mouse embryos (129 × NMRI). The age of the embryos was calculated by defining the day of the vaginal plug as day 0. Transfilter cultures were prepared using 11-d-old metanephric mesenchyme which was microsurgically separated from the ureter bud. Mesenchymes were placed on Nuclepore filters and cultured with spinal cord on the opposite side of the filter. The spinal cord was used as a heterologous inducer of development as described by Grobstein (1955, 1956). All cultures were carried out at 37°C and 5% CO_2 in a water saturated environment in Dulbecco's modified minimum essential medium as the basal medium (Vestweber et al., 1985; Klein et al., 1988) supplemented with 10% fetal calf serum.

Antibodies

Affinity-purified rabbit antibodies (0.36 mg/ml) against the E3 fragment of mouse Engelbreth-Holm-Swarm tumor laminin was used as a marker for the carboxy-terminal portion of the laminin A-chain. Such antibodies have been previously shown to react specifically with the A-chain of laminin in immunoblots (Klein et al., 1988). The GoH3 monoclonal antibody is a rat IgG raised against mouse mammary tumor plasma membrane extracts and has been shown to react specifically with the α_6 integrin subunit (Sonnenberg et al., 1986, 1987; Hemler et al., 1988). Ascites fluid containing this

antibody (1 mg/ml) was used in all experiments at the dilutions indicated. Ascites fluid containing monoclonal antibody against polysialic acid (Rougon et al., 1986) and nonimmune serum were used as controls.

Immunofluorescence

Kidneys and transfilter cultures of metanephric mesenchymes were frozen in Tissue-Tek (Miles Laboratories Inc., Naperville, IL) and 5-μm sections were prepared for immunofluorescence. Sections were fixed in methanol at -20°C for 5 min, nonspecific protein binding was saturated by incubating the sections for 5 min in PBS containing 1% BSA, and incubations with antibodies were subsequently carried out at room temperature. GoH3 was diluted 1:100, while the antiserum against E3 was diluted 1:200, in PBS. Since the two antibodies were raised in different animal species it was possible to perform double stainings with the E3 antiserum and GoH3 throughout all experiments. Bound antibodies were visualized using goat anti-rat IgG conjugated with rhodamine and goat anti–rabbit IgG conjugated with FITC (Dianova GmbH, Hamburg, FRG) second antibodies. Slides were examined under a Zeiss axiophot microscope equipped with epifluorescent optics.

Functional Assays

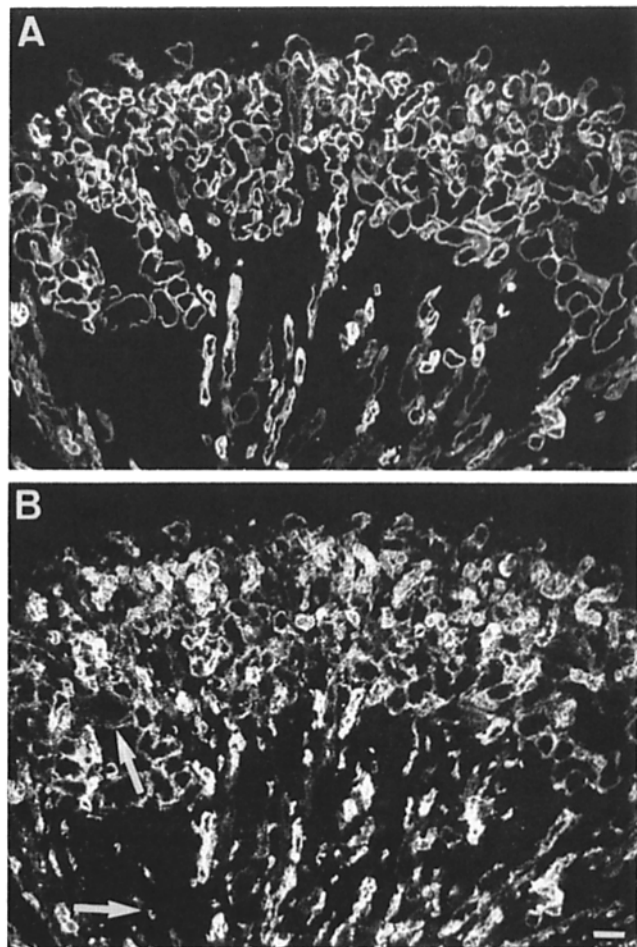Transfilter cultures were incubated in the presence of varying dilutions of

Figure 1. Colocalization by immunofluorescence of laminin A-chain (*A*) and the integrin α_6 subunit (*B*) in kidney of an 18-d-old mouse embryo. Note the extensive co-distribution of the A-chain and the α_6 integrin subunit in epithelial regions and the absence of A-chain and the integrin α_6 subunit in stromal tissue compartments. Tubules extending from the cortex to the medulla were also positive for laminin A-chain and the integrin α_6 subunit. The α_6 subunit but not the A-chain can be detected in blood vessels (*arrow*). Bar, 50 μm.
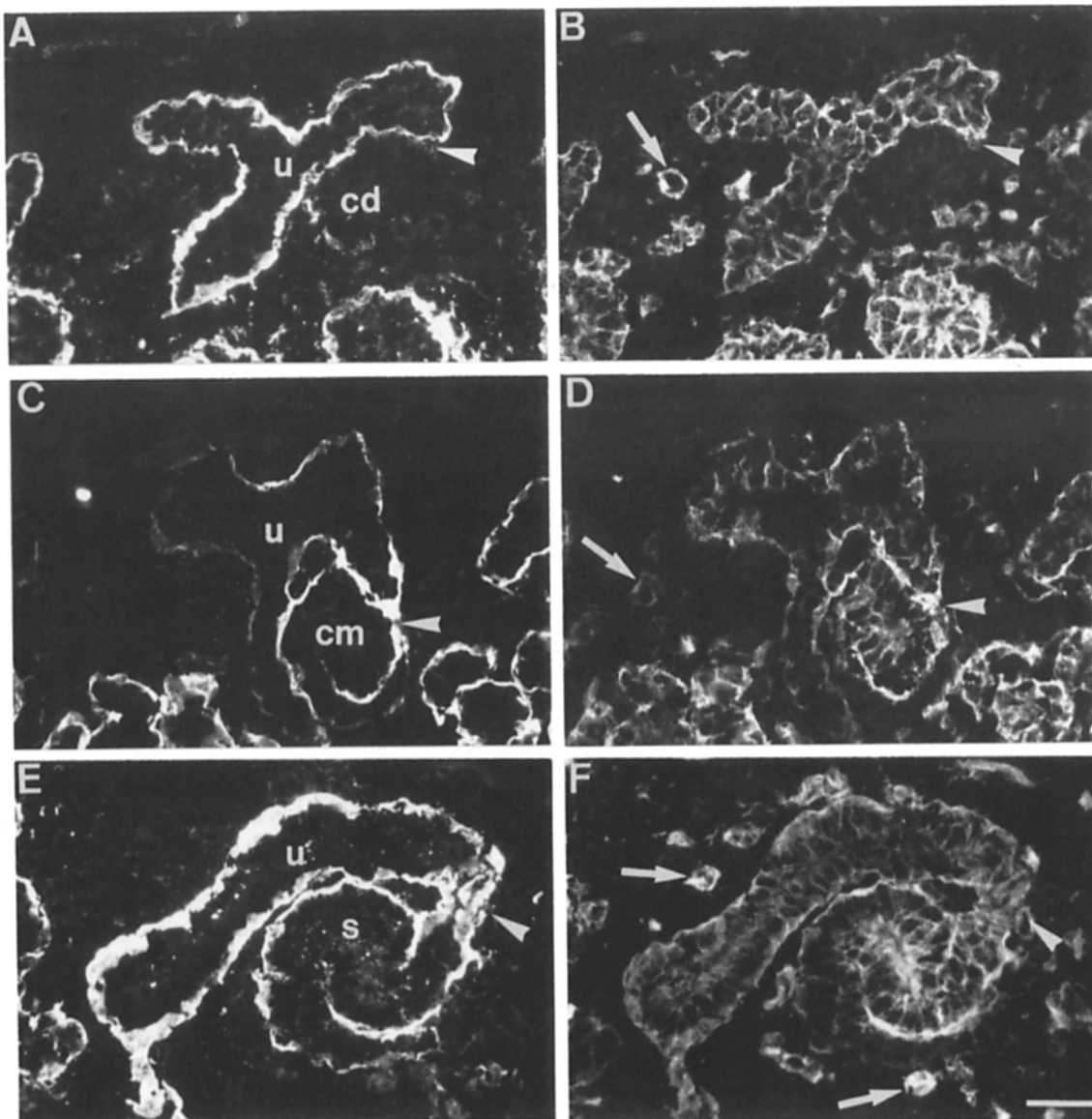
Figure 2. Colocalization of laminin A-chain (*left*) and the integrin α_6 subunit (*right*) at early stages of development of an individual nephron. The different stages in the development of epithelium from mesenchyme seen here below the tip of the right branch of the ureter epithelium (*u*) include: (*A* and *B*) the condensation-stage (*cd*); (*C* and *D*) the comma-shaped stage (*cm*); and (*E* and *F*) the S-shaped (*s*) stage. The area where the ureter tip merges with the mesenchymally derived epithelium is marked with an arrowhead. Note the weak expression of laminin A-chain and integrin α_6 subunit immunoreactivity in the early condensation stage (*A* and *B*); and the coexpression of the A-chain and the α_6 subunit in the comma-shaped stage (*C* and *D*). The areas around the epithelium, devoid of A-chain and the α_6 subunit, are the stromal tissue compartments. The endothelium of blood vessels (*arrows*) within the stroma express the integrin α_6 subunit (*B*, *D*, and *F*) but not the A-chain (*A*, *C*, and *E*). Bar, 50 μm.

ascites fluid containing GoH3. Control incubations were made in the same manner with ascites fluid containing antibodies to polysialic acid or without added antibodies. After 72 h of culture, samples were collected and frozen for immunocytochemistry or processed for histological examination as previously described (Vestweber et al., 1985; Klein et al., 1988). Tissues for histological examination were fixed in Zenker's solution and mounted in Technovit 7100 embedding plastic (Kulzer & Co. GmbH, Wehrheim, FRG). 4-μm sections were cut and stained with hematoxylin and eosin.

Results

Expression of Laminin A-Chain and α_6 Integrin Subunit in Developing Kidney

Three major tissue compartments are found in the develop-

ing kidney, mesenchyme, endothelium, and epithelium. Neither laminin A-chain nor the α_6 subunit were expressed in mesenchyme, but both were expressed in developing epithelia (Fig. 1, *A* and *B*). In addition, the α_6 subunit was expressed in endothelium (Fig. 1 *B*, Fig. 2, *B* and *F*). The apparent codistribution of the A-chain and the α_6 integrin subunit in the epithelial compartment suggested that these molecules are involved in epithelial cell development. The morphology of the different stages in epithelium development for individual nephrons has been well described (for example, see Osathanondh and Potter, 1963). The epithelium of the kidney originates from two cell lineages, the epithelial cells of the ureter and the nephrogenic mesenchyme. As a re-
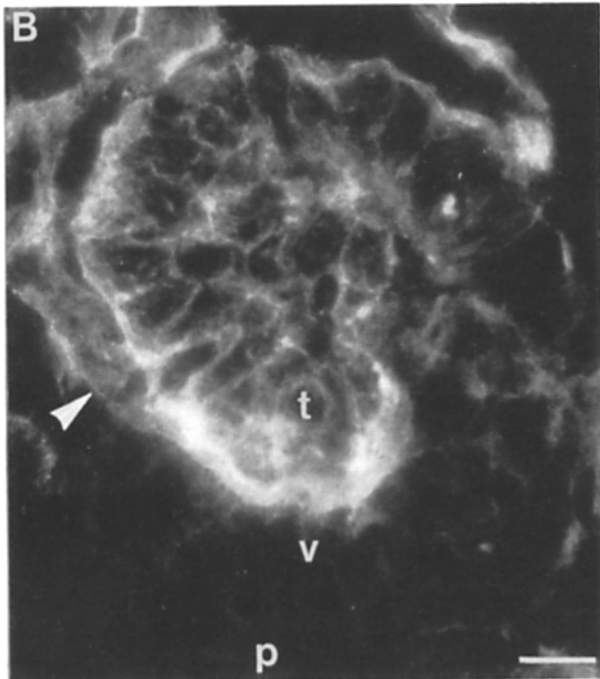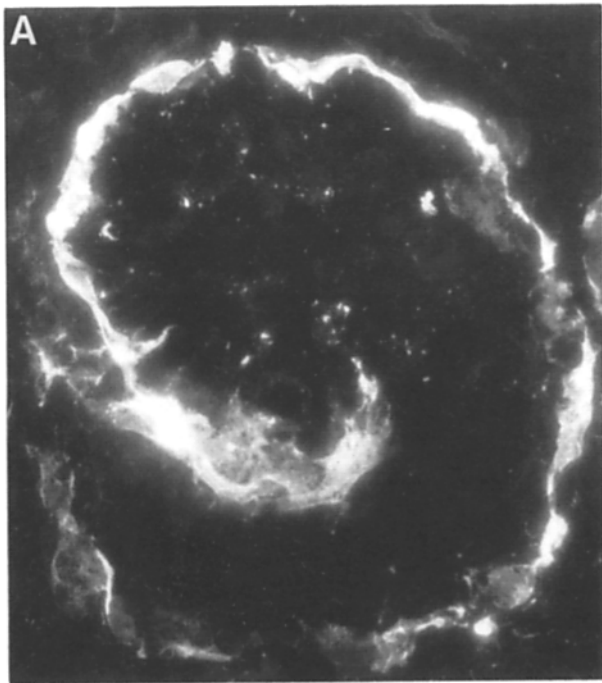
comma- and S-shaped structures. The epithelium of the S-shaped structures subsequently gives rise to the distal and proximal tubules and to the epithelial parts of the glomerulus. In the present study laminin A-chain and the α_6 subunit expression was investigated during this process using kidneys from 11- to 18-d-old mouse embryos.

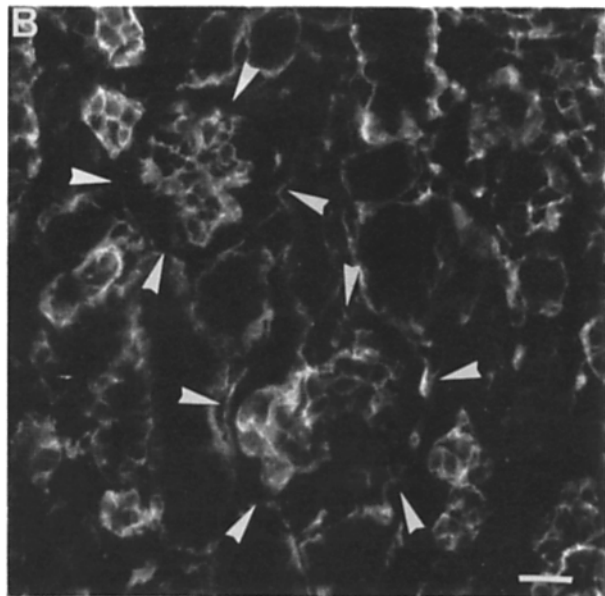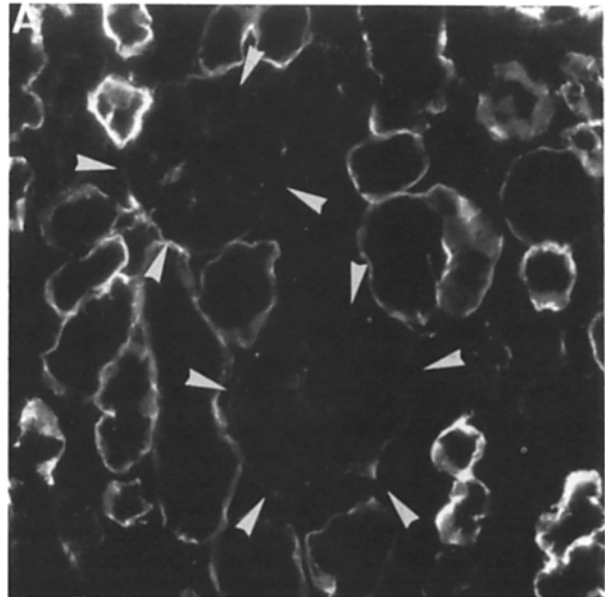Data from the embryos of different ages were similar. At

Figure 3. Details of laminin A-chain and α_6 integrin subunit expression in a developing comma-shaped tubule. Both the developing tubular and glomerular basement membranes express A-chain (*A*). The α_6 integrin subunit is clearly expressed on tabular epithelium (*t*) but not on parietal (*p*) or visceral (*v*) epithelium of the developing glomeruli (*B*). Arrowhead denotes endothelial cell. Bar, 10 µm.

Figure 4. Absence of A-chain (*A*) and presence of the integrin α_6 subunit (*B*) in the vasculature within developmentally advanced glomeruli. Endothelial and mesangial cells of subcortical glomeruli of 18-d-old mouse embryos express the α_6 subunit even though the A-chain of laminin cannot be detected in this region. Neither the visceral nor the parietal epithelium of the glomeruli express the α_6 integrin subunit. The only area with strong expression of A-chain in glomeruli was the basement membranes of the parietal epithelium. Note that the glomerular basement membrane located between podocytes and endothelium at this developmental stage expresses very little A-chain. Arrowheads denote the visceral epithelium of the glomeruli. Bar, 20 µm.

sult of their interaction, the ureter epithelium grows and branches extensively into the mesenchyme. At each tip of the ureter tree a part of the mesenchyme, in turn, responds to the ureter by differentiating into a new epithelium. Each ureter-induced conversion of mesenchyme results in the following stages: the induced cells first form condensates, then
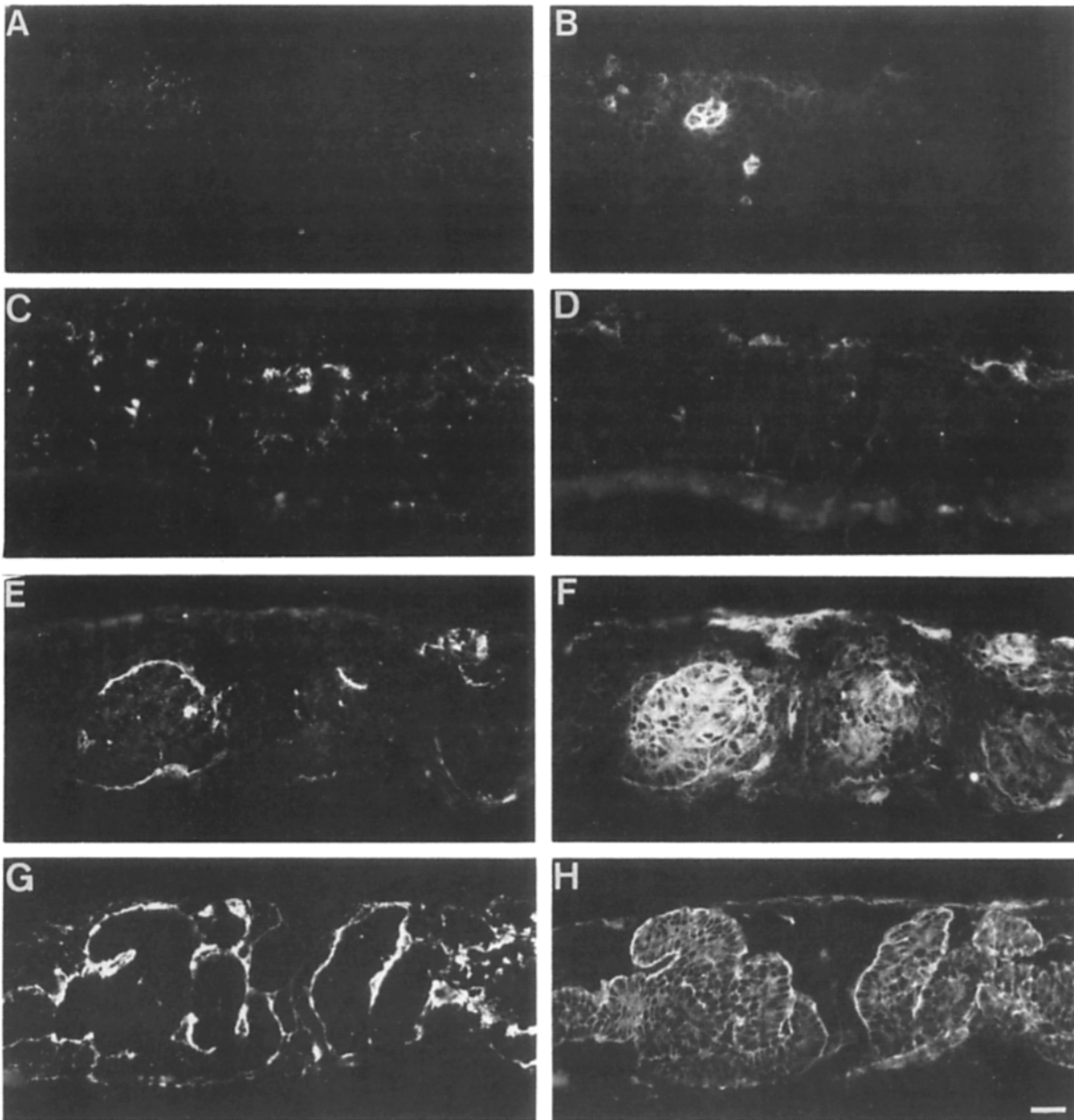
Figure 5. Co-appearance of laminin A-chain (*left*) and the integrin α_6 subunit (*right*) during the conversion of mesenchyme to epithelium in organ culture. Metanephric mesenchyme was separated from the ureter bud and cultured for 24 h without (*A* and *B*), or with spinal cord as an inducer tissue (*C* and *D*). No staining for A-chain (*A*) or the α_6 subunit (*B*) was apparent in the uninduced mesenchyme and expression of the α_6 subunit in (*B*) is restricted to blood vessels (*B*). In mesenchyme co-cultured for 24 h with spinal cord, weak expression of A-chain (*C*) and the α_6 subunit (*D*) was noted throughout the mesenchyme. In mesenchyme cultured for 36 h, cellular condensates formed that were bordered by laminin A-chain (*E*), while the surfaces of most of the cells constituting the condensates expressed the α_6 subunit (*F*). At 72 h of culture, polarized epithelial cells forming epithelial sheets are present. Note that the A-chain is restricted to the basal extracellular matrix of these sheets (*G*) and that the α_6 subunit is expressed on the entire cell surface of each epithelial cell with a slight enrichment basally (*H*). Bar, 20 μm.

the tips of the growing ureter epithelium, the extracellular areas adjacent to the basal surface of the epithelial cells were positive for laminin A-chain (Fig. 2 *A*), while the α_6 integrin subunit was uniformly distributed over the cell surface (Fig. 2 *B*). At this stage very little or no expression of either A-chain or α_6 subunit was seen in the mesenchymal condensates close to the tip of the ureter. In the comma-shaped stage, the A-chain began to appear in the basal extracellular

matrix (Fig. 2 *C*), and the α_6 subunit also became detectable on the surface of the cells constituting the comma-shaped bodies (Fig. 2 *D*). The next stage in epithelial cell development is the formation of S-shaped tubules. In both comma-shaped bodies of the S-shaped tubules there was a polar distribution of the laminin A-chain in the basement membrane underlying the newly forming epithelial cells (Fig. 2 *E*, Fig. 3 *A*). In contrast, the α_6 integrin subunit was expressed on

the entire cell surface of the epithelium, although a slightly stronger staining towards basal surfaces occurred (Fig. 2 F, Fig. 3 B). It is noteworthy that the basement membranes of the parietal epithelial cells of the glomerulus contain A-chain of laminin (Fig. 3 A) but very little α_6 integrin subunit (Fig. 3 B).

Although many epithelial tissue compartments expressed both the A-chain and the α_6 subunit, in some compartments the A-chain did not occur even though the α_6 subunit was expressed. Most notable was the absence of staining for laminin A-chain in endothelial cell basement membranes, even though an intense α_6 integrin expression was seen on the surface of the endothelial cells. This occurred in all age kidneys studied and was particularly well illustrated in the central area of glomeruli, composed entirely of endothelial and mesangial cells, and lacking mesenchyme (Fig. 4, A and B). The speckled pattern of immunofluorescence for the α_6 subunit in the nonepithelial compartment surrounding the developing tubules (Fig. 2, B, D, and F) also represents staining of endothelial cell surfaces.

At more advanced stages of ureter epithelium development, as occurs in 16- or 18-d-old embryos, A-chain immunoreactivity was no longer detectable. Yet the α_6 subunit continued to be expressed on ureter cells at all stages investigated, but was weaker than in the developing tubules. The intensity of staining for α_6 decreased progressively with ureter development but remained detectable on the epithelial cells of the ureter in adult kidneys. The A-chain of laminin and the α_6 subunit also continued to be present on the tubules of the adult kidney as has been previously described (Ekblom et al., 1990; Sonnenberg et al., 1990b).

Coappearance of Laminin A-Chain and α_6 Integrin Subunit during Conversion of Mesenchyme to Epithelium in In Vitro Culture

In the in vitro culture system of Grobstein (1956) the metanephric mesenchyme, isolated free from the ureter, is induced to differentiate by the embryonic spinal cord. In such co-cultures it is possible to follow the development of the tubules without the complication of the ingrowing ureter which morphologically appears similar to the developing tubules. Consequently, the transfilter cultures provide an ideal model for the investigation of whether there is a coordinated appearance of the A-chain and the α_6 integrin subunit during the course of development of kidney tubules.

No detectable staining for the A-chain of laminin was seen in mesenchyme cultured for 24 h in the absence of spinal cord (Fig. 5 A). Similarly, no staining for α_6 was evident in the same uninduced mesenchyme (Fig. 5 B). However, α_6 was expressed on the few endothelial cells of the blood vessels (Fig. 5 B), occasionally initially present in the in vitro cultures. After 24 h of culture in the presence of spinal cord, a weak and punctate staining pattern for both A-chain and α_6 was apparent throughout the mesenchyme (Fig. 5, C and D). More definite morphological signs of tubule formation were evident at 36 h of culture when areas of condensed mesenchyme began to form. In a few of these condensates, A-chain appeared in a punctate pattern throughout the forming condensates with the α_6 subunit located on the surfaces of the same cells (Fig. 5, E and F). The second type of expression pattern for A-chain was seen when the developing epithelial cells began to polarize. In such areas the staining

for A-chain became concentrated in the extracellular space towards the basal side of the peripheral cells of the condensate (Fig. 5 E). In contrast, the α_6 subunit was strongly expressed both on surfaces of cells in the middle and central parts of the condensates (Fig. 5 F). At 72 h of culture, polarized epithelial cells were clearly present, with A-chain restricted to the basal extracellular matrix (Fig. 5 G) and the α_6 subunit on the entire epithelial cell surface but enriched basally (Fig. 5 H).

Partial Inhibition of Tubule Formation by Antibodies to α_6 Integrin Subunit

Antibodies to the carboxy-terminal portion of the laminin A-chain inhibit the formation of epithelial tubules in the transfilter culture of metanephric mesenchymes, presumedly by inhibiting the interaction between cells and the laminin (Klein et al., 1988). If so, an antibody to a receptor for this biologically active part of the laminin molecule should have similar effects. The effect of the α_6 integrin specific antibody (GoH3) on tubule formation in transfilter cultures of metanephric mesenchyme was therefore tested.

Metanephric mesenchymes were cultured for 72 h in the presence of various dilutions of ascites fluid (1:12.5–1,000) containing the GoH3 antibody (1–80 μg/ml). The cultures were induced for 24 h with spinal cord, which was subsequently removed to ensure adequate penetration of the antibody into the tissue. Control cultures were performed in the same manner with ascites fluid containing a polysialic acid antibody in order to test for nonspecific effects of ascites fluid, or without added antibodies. To check whether antibodies were able to penetrate the tissues, direct immunofluorescence was performed on cryostat sections of tissues after 72 h of culture and staining was found within all tissues (not shown).

After 72 h of culture, well-polarized epithelial tubules were evident in control cultures incubated without antibodies, or with ascites fluid containing antibodies to polysialic acid (Fig. 6 A). Similarly, dilutions of 1:1,000–1:100 of the GoH3 antibody (1–10 μg/ml) had no effect on the development of tubules in the transfilter cultures. However, at dilutions of 1:50, 1:25, and 1:12.5 (20–80 μg/ml) of the GoH3 antibody, inhibition of tubule formation was evident. No difference in the degree of inhibition was evident between these different dilutions of GoH3. In four separate experiments at concentrations of 20–80 μg/ml constituting 36 different filters supporting mesenchymes, 27 showed partial inhibition of tubule formation (Fig. 6 B) while in the other nine complete inhibition occurred (Fig. 6 C). In the latter case, no areas of condensation of the mesenchyme or subsequent tubule formation were evident, but the mesenchyme was not without form and Fig. 6 C shows that a compact structure resulted. When partial inhibition of tubulogenesis occurred, the tubules that did form were indistinguishable from those in control cultures. However, the number of tubules that formed were notably fewer ($\sim$10–25%) than in control cultures and they occurred in discrete areas. The large areas in which no tubules were found contained no definite sign of mesenchyme condensation. The GoH3-treated cultures frequently became thicker, suggesting that the cells tended to pile up on each other although they could not form organized tubuli. In mesenchyme cultured in the presence of GoH3 some tissue necrosis was evident (Fig. 6 B).
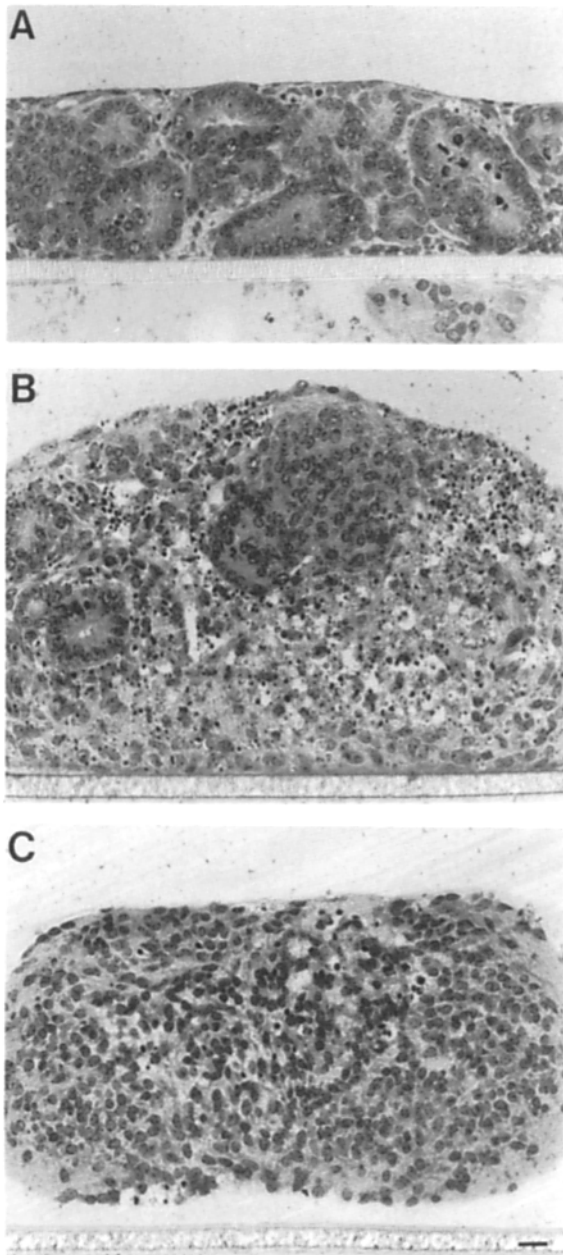
Figure 6. Perturbation of epithelial cell development by antibodies against the integrin α_6 subunit. Histological sections of mesenchyme cultured in the presence of either antibodies against the poly-sialic acid unit (*A*) or antibodies against the α_6 subunit (*B* and *C*). Mesenchyme was grown in transfilter culture for 24 h with spinal cord that was subsequently removed, and the cells cultured for a further 48 h before processing for histology. Note the well-formed tubules in cultures incubated in the presence of anti-polysialic acid unit at dilutions of 1:25, and partial (*B*) or complete inhibition (*C*) of tubulogenesis with antibodies against the α_6 at the same dilution. Some of the more organized areas in *B* may be clusters of glomerular podocytes rather than tubular cells. Note that the explants treated with antibodies against the α_6 subunit show an increased thickness. Bar, 20 μm.

Discussion

During kidney development nonpolar mesenchymal cells convert into epithelium as a result of an inductive tissue in-

teraction (Grobstein, 1955, 1956). The A-chain of laminin, one of the laminin chains produced by developing epithelial cells, may be important for this conversion as an autocrine stimulator of cell polarization (Klein et al., 1988). The present study demonstrated a marked coappearance of the α_6 integrin subunit and the A-chain of laminin during conversion of mesenchyme to epithelium. Furthermore, application of the anti-α_6 integrin receptor antibody consistently resulted in partial or complete inhibition of cell polarization in organ culture. The results presented here, together with previous organ culture data (Klein et al., 1988) and cell attachment studies (Sonnenberg et al., 1990 *a,b*; Aumailley et al., 1990*b*), suggest that the laminin of developing kidney tubules stimulates cell polarization by binding through fragment E8 to an integrin receptor possessing an α_6 subunit.

The conversion of mesenchyme into epithelium in the kidney involves several distinct steps. One early event is the induction of differentiation of the metanephric mesenchyme. During in vivo development induction is stimulated by the tips of the ingrowing ureter, and in vitro the same differentiation can be induced by tissues such as the embryonic spinal cord (Grobstein, 1956). One consequence of induction is an increased cell–cell adhesion which is followed by a polarization of cells to form an epithelium with a lumen. This development is accompanied by changes in the expression of several adhesion proteins (for recent reviews, see Ekblom, 1989; Bacallao and Fine, 1989). Analysis of these steps during in vivo development by immunofluorescence revealed that A-chain of laminin and the α_6-chain of an integrin receptor occurred on the same tissue compartments. Neither antigen was expressed in undifferentiated mesenchyme cells but both were present in the condensing mesenchyme, in the comma-shaped bodies indicative of the initiation of cell polarization, and in the ensuing epithelial kidney tubules. The major difference between the staining patterns obtained with the antiserum to the A-chain and α_6-specific antibody was apparent at the subcellular level and only after overt tubule formation. In such cases, laminin A-chain was located on the basal surfaces of the epithelial cells of the developing tubules, in the basement membrane, while α_6-chain was found on the entire cell surface but was enriched towards the basal surfaces of the cells. This basal enrichment, however, was seen only in developing tubules, and in the ureter α_6-chain was found uniformly on the entire surface of the epithelial cells.

The pattern of α_6 expression of the kidney sections and its co-distribution with laminin is similar to that described for other mouse tissues in which epithelial ducts or sheets form. However, previous studies were performed with antibodies to the whole laminin molecule (Sonnenberg et al., 1986) and therefore did not distinguish whether α_6 co-distributes with laminin A- or B-chains. The in vivo studies reported here clearly show a co-distribution of α_6 and the laminin A-chain in the epithelial cells during conversion of mesenchyme to kidney tubules. To define more precisely when the A-chain of laminin and α_6 first appear on the kidney tubules and whether their appearance was coordinated, conversion of mesenchyme into epithelium was studied in vitro (Grobstein, 1956). In such cultures epithelial cell polarization begins after 36 h of in vitro development and the appearance of the A-chain of laminin and the α_6 integrin subunit was coordinated at onset of epithelial morphogene-

sis. At 36 h of culture, when the mesenchyme began to form condensates, strong A-chain and α_6 integrin subunit staining was seen but was restricted to condensed areas. As the developing epithelial cells began to polarize the distribution of A-chain became increasingly polarized and enriched towards the basal surfaces of cells, a pattern that persisted in the fully formed tubules. In contrast, the α_6 continued to be expressed on all surfaces of the cells also at advanced developmental stages, with a slight enrichment towards the basal side of the cells.

The coordinated appearance of A-chain of laminin and α_6 integrin subunit in epithelial cells of developing kidney tubules, and their absence in mesenchyme that expresses laminin B-chains, is consistent with a role for α_6-chain as part of an epithelial cell laminin receptor. More direct evidence of such a role was obtained by testing whether the receptor antibody could perturb epithelial cell development in organ culture of the metanephric mesenchyme. When mesenchyme induced to differentiate by spinal cord was cultured in presence of the α_6-specific antibody at concentrations >20 μg/ml, either partial or complete inhibition of tubule formation consistently occurred. Although the concentration required to inhibit kidney tubule development is higher than those required to inhibit cell attachment in vitro (Sonnenberg et al., 1990a,b; Aumailley et al., 1990b), this result is striking because of the failure to inhibit kidney tubule development by antibodies against other cell surface adhesion proteins. The lack of effect of anti–polysialic acid antibodies even at low dilutions (1:25) is noteworthy since it has been suggested that the polysialic acid units could be important for early development of kidney tubules (Roth et al., 1987). During conversion of the kidney mesenchyme to epithelium, the epithelial cell adhesion molecule uvomorulin has a cellular distribution similar to that of the α_6 integrin subunit. Like the α_6 integrin subunit, uvomorulin first appears at the condensation stage and is later expressed on tubular epithelium but not on glomerular epithelium, and yet high concentrations (650 μg/ml) of antibodies to uvomorulin have failed to inhibit tubule morphogenesis (Vestweber et al., 1985).

The similarity of the effects of antibodies to the integrin receptor chain (α_6) and the laminin fragments (E8 or E3) (Klein et al., 1988) on in vitro kidney tubule development suggest that the E8 fragment of laminin controls cell polarization via an integrin receptor containing the α_6 subunit. The α_6-chain recognized by the GoH3 antibody has been shown to exist in combination with β_1 and β_4 subunits (Sonnenberg et al. 1988a; Hemler et al., 1989), and the $\alpha_6\beta_1$ integrin receptor has recently been shown to be a specific receptor for the E8 fragment (Sonnenberg et al., 1990a). Current data therefore raise the possibility that the receptor for laminin on the epithelial cells of developing kidney tubules is an $\alpha_6\beta_1$ integrin. One other possible laminin receptor is a 67–70-kD protein, the mRNA of which has been shown to be present in the kidneys of 16-d-old embryos. However, the cell types in the developing kidney expressing the mRNA could not be determined (Laurie et al., 1989). The 67–70-kD receptor has been implicated in binding to the P1 fragment of laminin (Graf et al., 1987), but antibodies to this part of the laminin molecule do not effect kidney tubule development (Klein et al., 1988). Consequently, it is unlikely that the 67–70-kD receptor plays a role in laminin-mediated stimulation of epithelial cell polarization during kidney development.

Although a marked co-distribution of the A-chain of laminin and the α_6 integrin subunit was seen in developing tubules, endothelial cells and some epithelial cells (ureter) did not express the A-chain even though they expressed the α_6 integrin subunit. It was noted that α_6 was not polarly expressed in endothelial cells and it is possible that the receptor recognized by GoH3 on these cells is not associated with an extracellular ligand. One possibility is that integrin receptors containing the α_6 subunit are also involved in direct cell–cell interactions in some endothelial or epithelial sheets, as has recently been shown for some integrins (Kaufmann et al., 1989; Carter et al., 1990; Larjava et al., 1990). Alternatively, it may be that the A-chain of laminin is masked in endothelial cell basement membranes, thereby rendering it indetectable by immunofluorescence. However, we have failed to detect the mRNA for A-chain in developing blood vessels by in situ hybridization and it is possible that the basement membranes of endothelial cells contain A-chain variants (Ekblom et al. 1990). The existence of laminin isoforms is now well documented (Edgar et al., 1988; Klein et al., 1988; Hunter et al., 1989; Paulsson and Saladin, 1989; Ekblom et al., 1990) and it is conceivable that they contain novel biologically active sites different from the carboxy-terminal portion of the laminin A-chain. Thus, both the laminin receptor and the laminin of endothelial cells probably differ in subunit composition from those found in developing tubules. It is already known that the $\alpha_6\beta_4$ receptors do not bind to the E8 fragment of laminin (Sonnenberg et al., 1990a,b) and recent studies on the human embryonic kidney suggest that the different tissue compartments of the embryonic kidney indeed express different types of integrins (Korhonen et al., 1990). Clearly, it will now be interesting to characterize the β subunits of the integrin complexes present on the endothelial and epithelial cells of the mouse kidney.

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