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Basement Membrane Complexes with Biological Activity

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ABSTRACT: We have studied the reconstitution of basement membrane molecules from extracts prepared from the basement membrane of the EHS tumor. Under physiological conditions and in the presence of added type IV collagen and heparan sulfate proteglycan, gellike structures form whose ultrastructure appears as interconnected thin sheets resembling the lamina dense zone of basement membrane. The major components of the reconstituted structures include laminin, type IV collagen, heparan sulfate proteoglycan, entactin, and nidogen. These components polymerize in constant proportions on reconstitution, suggesting that they interact in defined proportions. Molecular sieve studies on the soluble extract demonstrate that laminin, entactin, and nidogen are associated in large but dissociable complexes which may be a necessary intermediate in the deposition of basement membrane. The reconstituted matrix was biologically active and stimulated the growth and differentiation of certain cells.

Connective tissues contain different species of collagens, glycoproteins, and proteoglycans (Eyre, 1980; Bornstein & Sage, 1980; Kleinman et al., 1981; Hay, 1982). These macromolecules form the matrix structures that contribute to the physical characteristics of tissues as well as provide unique substrates for the resident cells. For example, the matrix produced by cultered fibroblasts consists of a dissociable complex of type I collagen, fibronectin, and heparan-containing and chondroitin sulfate containing proteoglycans (Hedman et al., 1983). Similarly, Schubert & LaCorbiere (1980) demonstrated the presence of complexes containing collagen, proteglycan, and glycoprotein in the media of cultured myoblasts and neural retinal cells. These complexes which they termed adherons also supported the attachment of cells (Schubert & LaCorbiere, 1982; Schubert et al., 1983).

In the present paper, we examine the macromolecular complexes involved in the formation of basement membranes. Basement membranes are thin but continuous sheets that separate epithelium from stroma and surround nerves, muscle fibers, smooth muscle cells, and fat cells (Kefalides, 1973; Vracko, 1974; Timpl & Martin, 1982; Laurie et al., 1983). Basement membranes contain type IV collagen (Kefalides, 1973; Orkin et al., 1976), the glycoproteins laminin (Timpl et al., 1979; Chung et al., 1979), entactin (Carlin et al., 1981), and nidogen (Timpl et al., 1983), and heparan sulfate proteoglycans (Kanwar & Farquhar, 1979; Hassell et al., 1980, 1985). In various studies, these materials show a codistribution (Leivo et al., 1982; Hayman et al., 1982; Laurie et al., 1982, 1984b) both within the lamina densa and within its extensions across the lamina lucida. Using electron microscopy, the components appear as a network of 5-nm-wide cords (Laurie et al., 1984), and by using electron microscopy, their codistribution suggests that the formation of basement membrane occurs through their interactions. Type IV collagen molecules form intermolecular disulfide bonds and associate in a continuous network (Timpl et al., 1981; Veis & Schwartz, 1981; Fessler & Fessler, 1982; Bächinger et al., 1982; Yurchenko & Furthmayr, 1984) which can be visualized in basement membranes digested with plasmin (Inoue et al., 1983).

Various components of the basement membrane are known to interact with each other. In vitro studies with purified components show that laminin binds through its short chains to native but not to denatured type IV collagen and through a domain in its long chain to the heparan sulfate proteoglycan (Terranova et al., 1980; Woodley et al., 1983; Rao et al., 1983). Alone each of these basement membrane components is soluble. When these macromolecules, however, are mixed together in vitro, they form a floccular precipitate containing laminin, type IV collagen, and heparan sulfate proteoglycan in a 1:1:0.1 molar ratio (Kleinman et al., 1983). However, this precipitate lacks the resiliency and consistency expected of basement membrane structures.

In this study, we have incubated extracts of the EHS tumor containing a mixture of proteins under physiological conditions and analyzed the components that interact and gel. These studies show that under physiological conditions certain components of the basement membrane including type IV collagen, laminin, heparan sulfate proteoglycan, nidogen, and entactin

interact in rather constant proportions to form a gel with lamellar structures resembling in width those in basement membranes. Under the conditions employed here, each of these components appears to be required for the reconstitution of the matrix. It is proposed that the components of the gel form supramolecular complexes, which may be intermediates in the formation of the matrix. The reconstituted matrix promotes the growth and differentiation of melanoma and certain other cells.

Experimental Procedures

Materials. Type IV collagen, laminin, and heparan sulfate proteoglycan were prepared from the EHS tumor (Timpl et al., 1979; Hassell et al., 1980, 1985; Kleinman et al., 1982). After the tumor tissue was washed in 3.4 M NaCl and 0.05 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, containing protease inhibitors (Orkin et al., 1976; Timpl et al., 1979), the basement membrane matrix was extracted with 0.5 M NaCl in 0.05 M Tris-HCl, pH 7.4. Laminin was isolated from the 0.5 M NaCl extract as previously described (Timpl et al., 1979). The residue of tumor tissue from lathyritic animals was extracted with 2.0 M guanidine in 0.05 M Tris-HCl, pH 7.4, followed by an extraction with the same buffer containing 0.005 M dithiothreitol to solubilize the type IV collagen (Kleinman et al., 1982). Low-density heparan sulfate proteoglycan was purified from 6.0 M urea extracts of the tumor by ion-exchange chromatography followed by cesium chloride density centrifugation and molecular sieve column chromatography (Hassell et al., 1980, 1985). Heparin was obtained from Sigma Chemical Co.

Unfractionated extracts of the basement membrane matrix were prepared by treating the tissue which had been washed with high salt with an equal volume (1 mL/g) of 2 M urea and 0.05 M Tris-HCl, pH 7.4, overnight at 4 °C and centrifuging at 10000*g* for 30 min. The residue was washed once with the same volume of buffer. Then the extract and wash were combined, dialyzed against 0.15 M NaCl in 0.05 M Tris-HCl, pH 7.4 (TBS), and centrifuged at 15000 rpm in a Sorvall centrifuge for 20 min to remove a small amount of insoluble material. The supernatant fraction was stored at −20 °C in small aliquots and used in the reconstitution assays described below. Using quantitative ELISA assays, this extract was found to contain laminin (3.5 mg/mL), type IV collagen (0.1 mg/mL), and heparan sulfate proteoglycan (0.1 mg/mL). Entactin, nidogen, and other minor components (Kleinman et al., 1982; J. R. Hassell, unpublished results) were also present. Entactin and nidogen were identified on the basis of their migration in sodium dodecyl sulfate (SDS) gels and cross-reactivity in Western blot analyses with antibodies obtained from A. Chung and R. Timpl, respectively. For column chromatography, the extract was dialyzed into 0.5 M NaCl and 0.05 M Tris-HCl, pH 7.4, and centrifuged at 40000 rpm for 1 h to remove insoluble material.

Reconstitution Assays. Gelation was carried out in a centrifuge tube to which 0.05–0.1 mL of the 2 M urea extract was added in physiological buffer. Purified components dissolved in 0.15 M NaCl and 0.05 Tris-HCl, pH 7.4, were added to the extract or were incubated together at the concentrations indicated. The final volume was made up to 0.5 or 1.0 mL with 0.15 M NaCl and 0.05 M Tris-HCl, pH 7.4, and the samples were incubated for 1 h at 35 °C. Insoluble material was isolated by centrifugation, and the pellets were dissolved in sample buffer and electrophoresed in either 5% of 7.5% acrylamide under reducing conditions (Laemmli, 1970). Each experiment was repeated a minimum of 3 times. The total amount of protein in the precipitate was determined by the Lowry procedure. The total amount of laminin was estimated by using the Lowry procedure but corrected for the relative amount of minor components present using scans of the gels. The amount of type IV collagen incorporated into the gel was quantitated by using ^{14}C-labeled type IV collagen, and heparan sulfate proteoglycan incorporation was quantitated by using [^{35}S]sulfate-labeled material of known specific activities in parallel experiments. Estimates of the amount of nidogen and entactin in the gel were obtained by scanning negatives of photographs of the gels in a Helena densitometer and then related to the amount of laminin and total protein in the gel.

Rotary Shadowing. The 2.0 M urea extract equilibrated in 0.5 M NaCl and 0.05 M Tris-HCl, pH 7.4, was placed on a Sepharose 4B column. An aliquot (30 μL) of the peak fraction (0.1 mg/mL) eluting from the column was diluted with 300 μL of 0.155 M ammonium acetate, pH 7.4, and 600 μL of glycerol. For rotary shadowing, the mixture was sprayed onto mica, shadowed with platinum–palladium, carbon coated, and examined in a JEOL 100C electron microscope.

Ultrastructure of Reconstituted Components. The gel was prepared essentially as described above. Briefly, 0.2 mL of the extract was incubated alone or in the presence of type IV collagen and heparan sulfate proteoglycan overnight at 35 °C. The gel was isolated by centrifugation at 10000 rpm for 10 min and then fixed in 2.5% glutaraldehyde, treated with 1% OsO_4, block-stained with 2% uranyl acetate, and dehydrated. The gel was then processed through Epon for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined in a JEOL 100 C electron microscope. Thin sections of rat kidney tubule basement membranes were obtained as previously described (Laurie et al., 1984b).

Cell Culture. B16C3 cells were cultured either directly on tissue culture plastic or on a 1-mm-thick basement membrane gel in a mixture of F12 medium and Dulbecco's modified Eagle's medium (lacking phenol red for visualization of the pigmentation of the cells) containing glutamine, antibiotics, 20 mM tyrosine, and 5% fetal calf serum. After 1 week, the cells were photographed.

Results

The assembly of basement membrane components was analyzed by using purified basement membrane components as well as unfractionated extracts of basement membrane. Purified type IV collagen, laminin, and heparan sulfate proteoglycan formed a flocculent precipitate when incubated under physiological conditions for 1 h at 35 °C (Kleinman et al., 1983). In contrast, a gel formed when urea extracts of basement membrane are dialyzed against physiological saline and then warmed to 35 °C for 1 h. The components of the gel were isolated by centrifugation and examined by SDS gel electrophoresis. The amount of laminin, entactin, and nidogen present in the gel was found to increase as increasing amounts of type IV collagen were added until some 50% of the material in the extract was incorporated into the gel (Figure 1A, C). Heparan sulfate proteoglycan also caused increasing amounts of basement membrane comonents to precipitate (Figure 1B). Separation by gel electrophoresis and determination of the relative amounts of major components in the gel by scanning the negatives of the gels indicated that constant ratios of laminin, entactin, and nidogen are obtained in the presence of added type IV collagen (Figure 1C) or of heparan sulfate proteoglycan (data not shown). When both type IV collagen (150 μg) and heparan sulfate proteoglycan (10 μg) were added to the extract, up to 80% of the protein present was incorporated into the gel (data not shown). Since the smaller chains

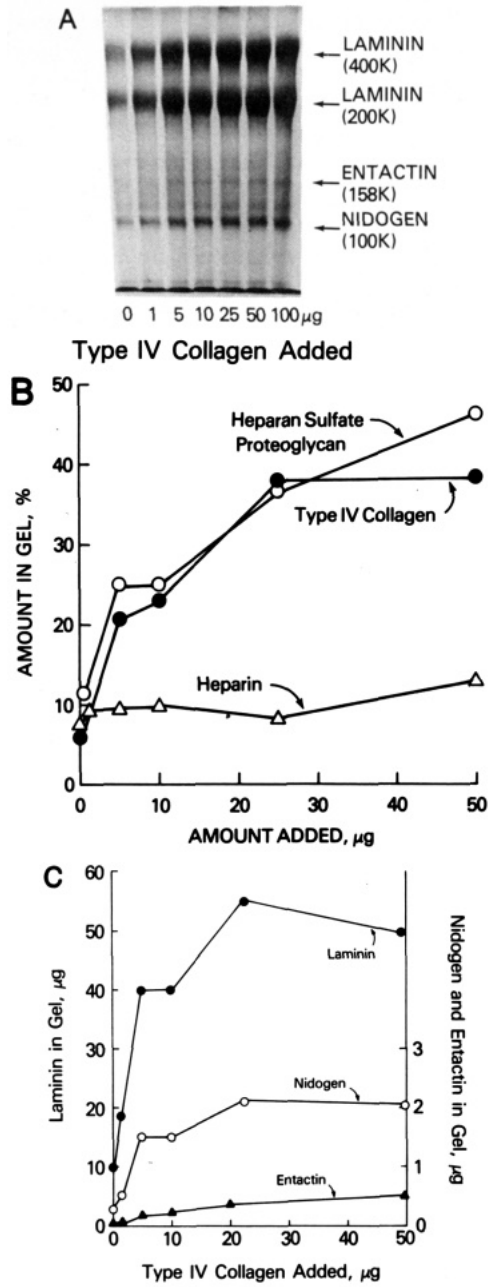

FIGURE 1: Effect of type IV collagen, heparan sulfate proteoglycan, and heparin on the gelation of basement membrane components from the basement membrane extract. Increasing amounts of each component were added to 100 μL of the extract and incubated for 1 h at 35 °C in 0.15 M NaCl and 0.05 M Tris-HCl, pH 7.4. The samples were then centrifuged, and the pellet was dissolved in sample buffer. Equal aliquots of the samples were electrophoresed in 5% acrylamide, fixed, and stained with Coomassie blue. (A) Effect of type IV collagen on the amount of protein in the gel. (B) Quantitative effects of type IV collagen, heparan sulfate proteoglycan, and heparin on the amount of protein present in the gel. The Lowry assay was used to determine the amount of protein. (C) Effect of type IV collagen on the amount of laminin, nidogen, and entactin in the gel. Densiometric scans of the SDS gels were used to determine the relative amounts of these components in the gel. The amounts of nidogen and entactin were estimated on the basis of their relative density and the total protein in the gel.

of laminin coelectrophoresed with the chains of type IV collagen and prevented its visualization in the SDS gel, we used ^{3}H-labeled type IV collagen of known specific activity in separate experiments to measure the amount of type IV collagen. Likewise, since heparan sulfate proteoglycan cannot be visualized in the gels, ^{35}S-labeled heparan sulfate proteoglycan was used. These studies showed that in a typical ex-

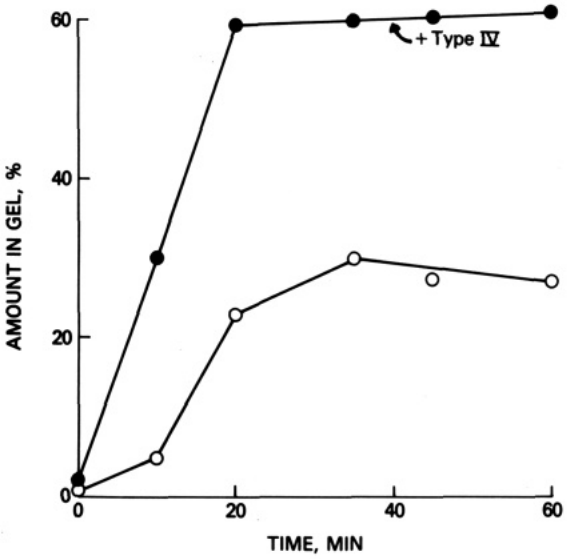

FIGURE 2: Effect of time and added type IV collagen on the gelation of the basement membrane extract. The conditions are similar to those described in the legend for Figure 1. This figure compares gelation in the presence (●) and absence (○) of type IV collagen (50 μg).

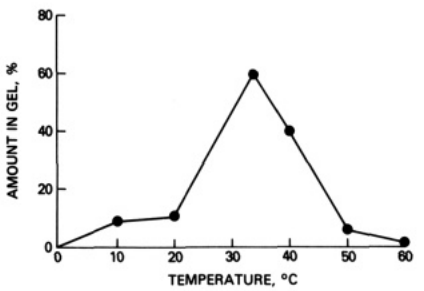

FIGURE 3: Effect of temperature on the gelation of extracts of basement membrane. The experiment was carried out in the presence of type IV collagen (50 μg) as described in the legend for Figure 2. Gelation was stopped by centrifugation at the times indicated.

periment laminin accounted for almost 60% (264 ± 56 μg) of the material in the gel, type IV collagen for 30% (125 ± 7 μg), heparan sulfate proteoglycan for less than 3% (8.3 ± 0.7 μg), nidogen for 5%, and entactin for less than 1%. These values are comparable to the proportions of laminin (15 mg/g wet weight), type IV collagen (8 mg/g), and heparan sulfate proteoglycan (1 mg/g) present in the EHS tumor (Kleinman et al., 1982). In contrast, supplementation of the extract with either type I collagen, fibronectin (data not shown), or heparin (Figure 1B) did not cause any increased precipitation, indicating that specific interactions are involved. Removal of the protein core of the proteoglycan by incubation overnight with 0.5 N NaOH destroyed its ability to induce polymerization, suggesting that the protein portion of the proteoglycan is involved in binding to other components (data not shown).

Under physiological conditions, the gelation process is complete within 20 min (Figure 2). The formation of the gel is strongly dependent on temperature with maximum polymerization at 35 °C (Figure 3). The lack of interaction at 50 °C suggests that thermal denaturation inactivates a critical constituent.

The stability of the gel to dissolution was examined by using various solvents. The gel was not dissolved by cold aqueous salt (0.15 or 0.5 M NaCl) but was partially dissolved by acidic solutions (43% solubilized) and almost completely dissolved in 2.0 M guanidine (97%) or in 2.0 M urea solutions. This suggests that the components are linked by relatively strong but noncovalent bonds. When the guanidine-dissolved gel was

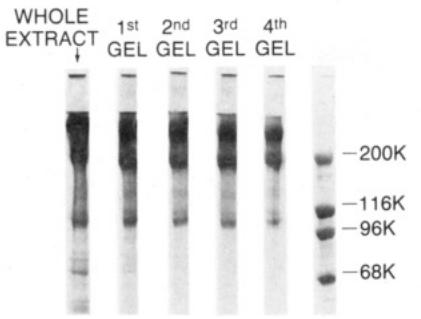

FIGURE 4: Ability of the basement membrane extract to regel following dissolution of the gel. The first lane, designated "whole extract", demonstrates the components in the starting material. The "1st gel" designates the components in the gel formed in the presence of type IV collagen. The material present in the gel formed in the absence of type IV collagen (not shown) was solubilized for 20 min in 2.0 M guanidine, dialyzed against 0.05 M Tris-HCl, pH 7.4, containing 0.15 M NaCl, and allowed to regel in the absence (not shown) and presence of type IV collagen (designated "2nd gel"). The cycle was repeated 2 additional times ("3rd gel" and "4th gel"). Shown are equal aliquots of the gels electrophoresed in a 5% acrylamide gel.

dialyzed against physiological buffers and warmed in the presence of type IV collagen, gellike structures were reconstituted. This process could be repeated several times with similar proportions of laminin, nidogen, and entactin depositing at each step as determined by SDS–polyacrylamide gels (Figure 4). Densiometric scans of the gels indicated that laminin and type IV collagen were 94% of the total material and nidogen and entactin represented 5% and 1%, respectively. In the presence of added type IV collagen, re-formation of the gel occurred more rapidly, and greater amounts of the components were deposited (data not shown).

We next determined whether soluble complexes of basement membrane components existed. When the urea extract was dialyzed free of urea and passed over a Sepharose 4B column in 0.5 M NaCl (associative conditions), laminin, nidogen, and entactin eluted in a major included peak (Figure 5A,B). When the material in the major included peak was pooled and rerun over the same molecular sieve column in 4 M guanidine (dissociative conditions), these components separated in the manner expected from their molecular weights (Figure 5A,C). These results indicate that there are strong but noncovalent bonds joining laminin, nidogen, and entactin in the complex. Rotary shadowing electron microscopy of the major included peak material confirmed the presence of soluble complexes (Figure 5A). The complexes involved the proteoglycan which appears as a large globule due to collapse of the heparan sulfate side chains in this kind of preparation (G. W. Laurie et al., unpublished results) surrounded by several laminin molecules. The nidogen and entactin molecules could not be distinguished in the electron micrographs but are known to be in the complexes from the electrophoresis study (Figure 5B).

The ultrastructure of the reconstituted basement membrane both with and without type IV collagen and heparan sulfate proteoglycan was examined. In the asbence of added type IV collagen and heparan sulfate proteoglycan, the gel consisted of numerous widely separated thin, filamentous aggregates (Figure 6a). The addition of type IV collagen (not shown) or of heparan sulfate proteoglycan plus type IV collagen (Figure 6b) resulted in the formation of thin sheets which were interconnected (Figure 6b) or were confluent. The individual segements of the network had an average width similar to that of the lamina densa of kidney tubule basement membrane (Figure 6c). However, unlike native basement membranes in which lamina densalike layers are arranged in parallel, such as, for example, the PYS tumor basement membranes

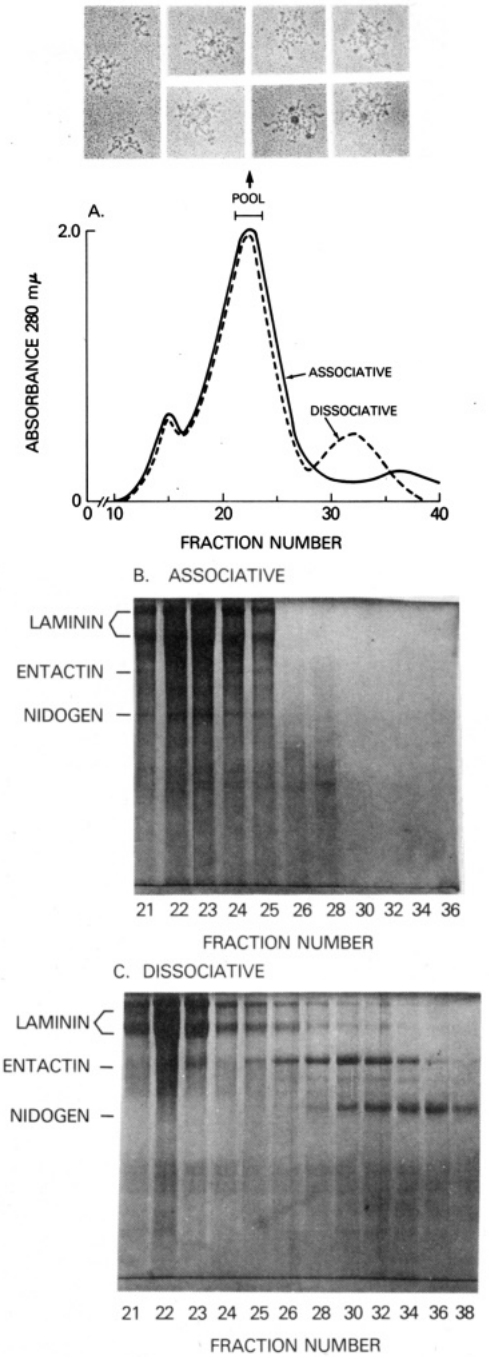

FIGURE 5: Sepharose 4B column chromatography of the 2.0 M urea extract. Two milliliters of the whole extract equilibrated in either 2 M urea, 0.15 M NaCl, and 0.05 M Tris, pH 7.4 (dissociative), or 0.5 M NaCl and 0.05 M Tris, pH 7.4 (associative), was placed on a Sepharose 4B column (2 × 60 cm) equilibrated in the corresponding buffer. (A) Aliquots of the designated fractions from the extract chromatographed in associative (B) or dissociative (C) conditions were analyzed by SDS–polyacrylamide gels. In addition, an aliquot of the material eluting from the column run under associative conditions was examined in the electron microscope by rotary shadowing (A). The electron micrographs show that the most common complex in the peak fractions involves a central heparan sulfate proteoglycan and numerous peripheral laminin molecules. Entactin and nidogen are not readily visualized in these complexes.

(Martinez-Hernandez et al., 1982) or Reichert's membrane (Inoue et al., 1983), the lamina densalike structures were interconnected and did not form parallel multilamellar structures. At very high power in the electron microscope, each segment could be resolved into 5-nm cords as previously described in other basement membranes (Inoue et al., 1983; Laurie et al., 1984).

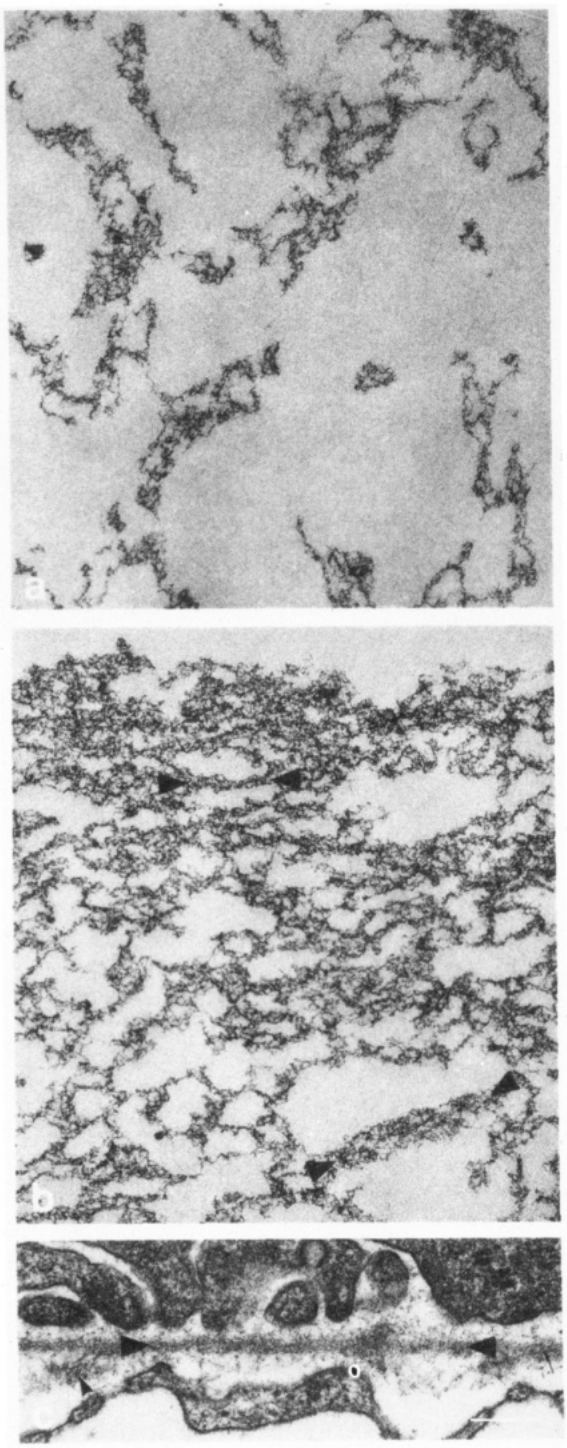

FIGURE 6: Electron micrographs of reconstituted gels and an authentic basement membrane. (a) Gel formed in the absence of added type IV collagen or heparan sulfate proteoglycan. The gel consists of dispersed segments with occasional interconnections. (b) Gel formed in the presence of added type IV collagen and heparan sulfate proteoglycan. The edge of the gel is at the top. The gel consists of an interconnected network; the network is made up of structures which are similar in width to the lamina densa part of native basement membranes. These lamina densalike structures vary somewhat in thickness. (c) Kidney tubule basement membrane from a 100-g rat. The basement membrane consists of the lamina lucida and lamina densa. Extensions from the lamina densa attach it to the cell membrane (arrowheads). Bar = 200 μm; 42750×.

The reconstituted basement membrane was used to coat the surfaces of bacteriological petri dishes and tested as a substrate for the growth and differentiation of various cells. Melanoma cells (B16C3) showed considerable differences in morphology when grown on the basement membrane gel as compared to

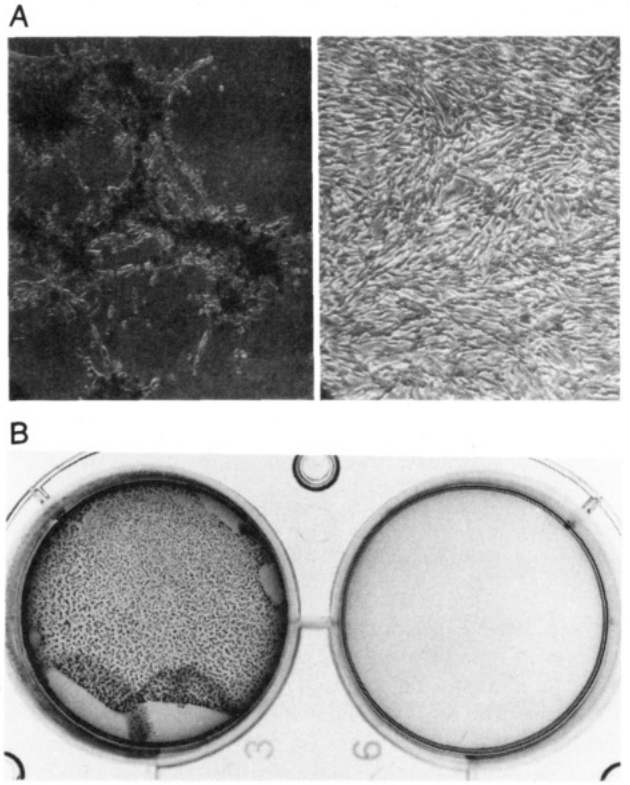

FIGURE 7: Effect of the basement membrane gel on the morphology and differentiation of B16C3 melanoma cells in culture. Sterile 2 M urea extract of the EHS tumors in 0.15 M NaCl and 0.05 M Tris-HCl, pH 7.4, was allowed to gel on the surface of a petri dish for 30 min at 37 °C. Then equal numbers of cells were plated onto the gel (left) or onto control tissue culture plastic dishes (right). After 1 week in culture in DMEM containing 20 mM tyrosine, gentamicin, glutamine, and 5% fetal calf serum, the cells were photographed. (A) Morphology and assessment of melanogenesis by the cells. (B) Direct view of the dishes. The gel at the edge has been deflected to show that the cells are attached to it.

tissue culture surfaces (Figure 7). Further, there was a much earlier and more extensive pigmentation of the cells on this substrate. Preliminary studies of other cells showed that endothelial cells formed tubelike structures on the gel (G. Grotendorst, unpublished results) and that hepatocytes survived longer on basement membrane gel substrates than on tissue culture plates or on type I collagen (L. Reid, unpublished results). In vivo, the basement membrane gel was found to promote peripheral nerve regeneration (Madison et al., 1985). Such studies indicate that the reconstituted basement membrane is a biologically active substrate which induces diverse cellular responses. Since it can support cell adhesion, growth, and differentiation beyond that reported for the individual components, it is likely that the reconstituted basement membrane gel contains these molecules in an active and authentic conformation.

Discussion

It is now well appreciated that, while collagens form the major structural element of extracellular matrices, glycoproteins and proteoglycans are also important constituents, regulating many other activities including cell–matrix interactions (Hay, 1982; Timpl & Martin, 1982). Different matrices contain different sets of these matrix proteins which together contribute to the unique physical and biological characteristics of tissues.

In studies on the constituents of basement membranes, we have observed that they form supramolecular complexes (Kleinman et al., 1983). The individual components, type IV

collagen, laminin, and heparan sulfate proteoglycan, are soluble whereas they precipitate when incubated in combination. These observations have led us to suggest that cooperative interactions are involved in their deposition into the basement membrane. This is not to say that the individual components cannot self-assemble. Type IV collagen molecules obtained from the media of cultured cells form tetrameric aggregates linked through the amino-terminal portion of the molecule (Bächinger et al, 1982; Fessler & Fessler, 1982). Dimeric components of type IV collagen such as used here will form a large open network, while laminin will also self-aggregate (Yurchenco & Furthmayr, 1984; Yurchenco et al., 1984). We presume, however, that these components are synthesized concurrently and that cooperative interactions may predominate. In addition, such interactions are not likely to be important in the initial interactions since prior incubation of the type IV collagen, under conditions where type IV self-assembles, does not affect the amount of material incorporated into the gel, the rate of gel formation, or the appearance of the gel (unpublished observations).

In this study, we have attempted to determine whether additional components are involved in the formation of basement membrane and whether these are specific and stoichiometric interactions. In these studies, the basement membrane of EHS tissues was extracted with a chaotropic solvent (2 M urea) to disrupt noncovalent interactions and to bring various glycoproteins and proteoglycans into solution. Only small amounts of type IV collagen are present in the extract since it is cross-linked by disulfide bonds and by lysine-derived cross-links. Some of the material in the extract formed a gel when the solution was dialyzed against physiological saline and brought to 37 °C. However, when type IV collagen was added to the extract, there was an increased deposition of laminin, nidogen, and entactin. The addition of type IV collagen plus heparan sulfate proteoglycan leads to a maximal incorporation of these compounds into a gellike structure containing interconnected thin sheets.

Experiments described here and previously suggest that these interactions are specific (Terranova et al., 1980; Woodley et al., 1983; Kleinman et al., 1983). For example, type I collagen cannot substitute for type IV collagen, nor fibronectin for laminin nor heparin for heparan sulfate proteoglycan. Such specificity is expected and parallels the known affinities of these components for binding to one another. In addition, there appears to be a stoichiometric relation in the interaction of these materials. When redissolved, the components of the gel reconstitute in the same proportions. Such behavior is expected if these interactions occur through a limited number of specific binding sites as suggested in studies on their interactions (Rao et al., 1983; Mai & Chung, 1984; Laurie et al., 1984a; Dziadek & Timpl, 1985). However, it is possible that nidogen and entactin and perhaps other minor components of the basement membrane may be involved in organizing the materials that precipitate into gellike and sheetlike structures.

Molecular sieve studies also supported the existence of supramolecular complexes which were dissociable in guanidine, and these complexes were visualized directly after rotary shadowing by electron microscopy. The major features of these complexes appear to be a central core of proteoglycan surrounded by several laminin molecules. Laminim alone does not form such complexes but will when incubated with heparan sulfate proteoglycan (G. W. Laurie et al., unpublished results). Nidogen and entactin could not be distinguished in these preparations perhaps because they are much smaller than the other constituents. Taken together, these studies indicate that laminin, nidogen, and entactin form defined complexes and that type IV collagen and heparan sulfate proteoglycan cause them to be incorporated into an insoluble gellike structure.

Supramolecular complexes of matrix components have been observed in other systems and have been shown to consist of procollagen, proteoglycan, and fibronectin (Schubert & LaCorbiere, 1980; Hedman et al., 1982; Schubert et al., 1983). Again, these materials are produced by the same cell and may even be secreted together. Thus, it is possible that supramolecular complexes of matrix components arise in a variety of sites and have a role in the formation of as well as in determining the composition of newly deposited extracellular matrices.

Since matrix substrates affect the behavior of cultured cells, we tested the growth and differentiation of cells on the reconstituted basement membrane. To date, we and others to whom we have supplied the gel have found rather strong effects on the growth and differentiation of cultured cells. As shown here, melanocytes aggregate into clusters and show an accelerated melanogenesis. Hadley et al. (1985) have found that dissociated embryonic Sertoli cells will reorganize in the gel into tubular structures which resemble in organization the original tissue. The gel also encourages the in vivo growth of neurons (Madison et al., 1985) and the in vitro growth and differentiation of Schwann cells (D. Carey and M. Todd, unpublished results) and liver cells (L. Reid, unpublished results). In general, epithelial cells assume a much greater polarity on this substrate then on plastic, collagen, laminin, or fibronectin substrates.

The ability of complex substrates to support cell growth and differentiation is well documented [see, for example, Dodson & Hay (1974), Rojkind et al. (1980), Gospodarowicz & Lui (1981), Lillie & MacCallum (1982), Mai & Chung (1984), and Wicha et al. (1982)]. Cells appear to have specific receptors not only for glycoproteins such as laminin and fibronectin but also for collagen and proteoglycans (Goldberg, 1979; Rubin et al., 1978; Kurkinen et al., 1984; Bernfield & Banerjee, 1982; Lattera et al., 1980). Presumably cells function best on their natural matrix with each cellular receptor bound to the appropriate matrix component.

Added in Proof

Since this paper was prepared, it has been found that nidogen has higher molecular weight forms similar in size to entactin. Thus, entactin and nidogen may be related proteins.

Acknowledgments

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