Monoclonal Antibodies to Laminin Reveal the Heterogeneity of Basement Membranes in the Developing and Adult Mouse Tissues

YU-JUI WAN, TSUNG-CHIEH WU, ALBERT E. CHUNG,* and IVAN DAMJANOV
Department of Pathology and Laboratory Medicine, Hahnemann University School of Medicine,
Philadelphia, Pennsylvania 19102; and * Department of Biological Sciences, University of Pittsburgh,
Pittsburgh, Pennsylvania 15260

ABSTRACT Two monoclonal antibodies raised against laminin isolated from a mouse parietal yolk sac cell line were used for immunohistochemical studies of basement membranes of the mouse embryo and various fetal and adult tissues. No immunoreactivity with either of the two monoclonal antibodies could be detected in the preimplantation-stage embryos, although it has been shown that these embryos contain extracellular laminin reactive with the conventional polyclonal antilaminin antibodies. Reichert's membrane in early postimplantation stages of development reacted with the monoclonal antibody LAM-I but not with the antibody LAM-II. However, from day 8 of pregnancy onward the Reichert's membrane reacted with both antibodies. Basement membranes of the embryo proper were unreactive with both monoclonal antibodies until day 12 of pregnancy. By day 14 some basement membranes of the fetal tissues became reactive with one or both monoclonal antibodies, whereas others remained still unreactive. In the 17-d fetus and the newborn mouse most of the basement membranes reacted with both monoclonal antibodies, whereas others still reacted with only one. Similar heterogeneity in the immunoreactivity of basement membranes of various tissues was noted in the adult mouse as well. These results indicate that the immunoreactivity of laminin in the extracellular matrix changes during development and that the basement membranes in various anatomic locations display heterogeneity even in the adult mouse.

Laminin or GP-2, a high molecular weight glycoprotein isolated initially from the extracellular matrix of mouse endodermal-like cells (1, 2), is one of the ubiquitous and constant intrinsic components of all epithelial basement membranes so far studied (3). Antibodies raised in rabbits against laminin isolated from mouse or rat yolk sac carcinomas react with basement membranes of normal murine tissues as well as with human basement membranes, indicating that the laminin produced by the neoplastic cells resembles the equivalent glycoprotein from normal tissues.

We have recently produced two monoclonal antibodies to laminin (4). These monoclonal antibodies react with the polypeptide structure of the 220,000-dalton component of laminin isolated from mouse M1536-B3 cells and also react with the basement membranes of some normal adult mouse tissues (4). However, one of these two antibodies does not react with human renal or other basement membranes sug-

gesting that the epithelial basement membranes of mouse and man differ if not in their composition then in steric and/or conformational arrangement of their constituent components, which render them reactive or unreactive with a given monoclonal antibody. Peters and Goldstein (5) have noticed similar differences with regard to the binding of lectin from Bandeiraea simplicifolia. This lectin reacts with mouse renal basement membranes but does not react with human renal basement membranes, suggesting that there are indeed differences between basement membranes of different species.

In the present study we have used the monoclonal antibodies to laminin to study basement membranes during mouse embryonic development. Extracellular matrix found in early mouse embryos contains laminin from very early stages of development onward (6–8). However, we show that the basement membranes of developing mouse embryos do not react with the monoclonal antibodies to laminin until

mid-gestation. This change in the immunoreactivity of the extracellular matrix suggests that the basement membranes undergo conformational and/or other structural modifications during embryonic and fetal development. Along the same lines, we also show a considerable heterogeneity of basement membranes in adult tissues as evidenced by their differential reactivity with the polyclonal and monoclonal antibodies to laminin.

MATERIALS AND METHODS

Animal Material: Embryos and fetuses obtained from outbred Swiss Webster mice were mated overnight. The day when the plug was found was labeled as day 0. Preimplantation-stage embryos were isolated in toto from the oviduct and uterus. Early postimplantation-stage embryos were isolated and handled together with deciduoma. Fetuses older than 12 d were isolated from fetal membranes and processed in toto or dissected into major anatomical portions. Organs were disected from adult mice and each of them was processed separately.

Antibodies and Sera: Rabbit antilaminin sera were prepared against highly purified laminin isolated from the mouse endodermal cells M-1536-B3 (9) or from a rat yolk sac carcinoma (10). The latter antibodies were kindly provided by Dr. A. Martinez-Hernandez (Hahnemann University, Philadelphia). The characterization and purification of these antisera were described in detail before (9, 10). Monoclonal antibodies LAM-I and LAM-II were prepared as described by Chung et al. (4). Pristane-primed nude mice were injected intraperitoneally with 10^6 hybridoma cells per mouse. The ascites fluid was tapped 7–10 d later and clarified by centrifugation. The clarified fluid was purified on DEAC-cellulose as described by Garvey et al. (11). The specificity of the antibody was determined by immunoblotting, immunoprecipitation, and enzyme-linked immunosorbent assay methods as previously described (4). In the immunoblotting to nitrocellulose filters, both antibodies reacted against the GP-2 subunit of laminin but not against entactin. A very slight but variable reaction was observed with GP-1 which could reflect either some cross-reactivity with the GP-1 subunit of laminin or the presence of a common antigenic determinant. Immunoprecipitation of tunicamycin-treated M1536-B3 cells resulted in the precipitation of the unglycosylated precursor bands of GP-2 which demonstrated that the antibody recognized the polypeptide backbone of the molecule. Finally, by enzyme-linked immunosorbent assay techniques, free laminin was found to block the binding of antibodies to laminin-coated plates. Antibody was also prepared by first growing hybridoma cells in Eagle's α-minimal essential medium obtained from Gibco (Grand Island, NY), which contained 10% horse serum, and then transferring the cells at a density of 10^6 cells/ml to serum-free α-minimal essential medium. The culture fluid was collected after 24–48 h and the antibodies were precipitated with ammonium sulfate at 50% saturation. The precipitate was redissolved in PBS and dialyzed against 100 vol of the same buffer overnight. The dialyzed sample was either stored frozen or lyophilized. The stock solutions of antibodies contained 3–5 mg protein/ml and were used diluted in PBS 1:30. This optimal dilution was determined by titration on sections of adult mouse kidneys.

Immunohistochemistry: Preimplantation-stage embryos were fixed in 4% paraformaldehyde at 4°C for 2 h, washed in PBS, and incubated in the primary antibody at room temperature as described before (8). In some instances the zona pellucida was removed with 0.5% pronase before incubation. The postimplantation embryos and fetuses and isolated organs from adult animals were placed into OCT (Lab Tech, Naperville, IN) and snap-frozen in methyl butane precooled to liquid nitrogen temperature. The specimens were sectioned in a cryostat, fixed in paraformaldehyde or acetone, and incubated in primary antibody as described (8). The binding of the primary antibody was

detected with fluorescein-conjugated goat anti-rabbit or rabbit anti-mouse IgG antiserum (Cappel Laboratories, Cochranville, PA). To prevent the nonspecific binding of anti-IgG antiserum to endogenous mouse immunoglobulin transmitted from the mother into the fetus and normally found in the visceral endoderm, we first incubated some sections of early postimplantation embryos and the yolk sac with unlabeled rabbit anti-mouse IgG serum for 1 h and then exposed them to the primary and secondary antibody.

To assess the effects of fixation on the immunohistochemical demonstrability of laminin, we used several additional fixatives. Thus the samples were fixed by immersion in −20°C methanol for 10 min or acetic ethanol solution (1% glacial acetic acid and 99% absolute ethanol) at 4°C for 20 min. Unfixed sections were also used. In an attempt to determine whether the antigenic determinants are masked in the tissue sections and thus inaccessible to the antibodies, some freshly frozen, cryostat-sectioned tissues were fixed in 4% paraformaldehyde and digested with the following enzymes: mixed glycosidases (10 mg/ml); chondroitinase ABC (1 U/ml) purchased from Miles Laboratories, (Elkhart, IN); 0.2% pepsin, 2% bovine testicular hyaluronidase, Clostridium perfringens neuraminidase (0.1 U/ml) purchased from Sigma Chemical Co. (St. Louis, MO). All the digestions were performed at room temperature for 20–30 min and were followed by a wash in cold PBS and an incubation procedure identical to the one described above. Control sections were incubated with the ascites fluid of the (P3-X63-Ag8) myeloma instead with the specific antibodies. Alternatively the primary antibody was deleted and the sections were incubated only in the secondary fluorescein isothiocyanate–labeled antibody.

RESULTS

The results are summarized in Tables I and II.

Preimplantation-stage Embryos

It has been conclusively shown that cells of cleavage-stage mouse embryos synthesize (7) and secrete laminin whose presence can be demonstrated immunohistochemically in the extracellular matrix from 8–16–cell stage of development onward (6, 8). Using the two monoclonal antibodies, we were unable to demonstrate any extracellular or intracellular immunoreactive laminin in cleavage-stage embryos, morulae, and early or late blastocysts. Identical results were obtained with fixed and unfixed embryos, as well as embryos permeabilized with Nonidet P-40. All the embryos from the eight-cell stage on contained extracellular material immunoreactive with the polyclonal antibodies to laminin.

Postimplantation-stage and Somitic Embryos (6–12 d)

The 6-d-old embryonic egg cylinder is enveloped by a fully developed Reichert's membrane that separates it from the surrounding deciduoma. The Reichert's membrane is intensely immunoreactive with the polyclonal antibody to laminin (Fig. 1a). This antibody also reacts with the basement membrane between the visceral endoderm and the ectoderm and the intercellular matrix of the deciduoma. Monoclonal antibody LAM-I reacted with the Reichert's membrane (Fig.

TABLE I

Immunoreactivity of Embryonic Structures with Monoclonal and Polyclonal Antibodies to Laminin

Antibodies	Cleavage-stage embryo*	6-d Embryo		8-d Embryo	12-d Embryo	
		Reichert's membrane	Basement membrane[‡]	Reichert's membrane	Reichert's membrane	Basement membrane[‡]
Monoclonal						
LAM-I	−	+	−	+	+	−
LAM-II	−	−	−	+	+	−
Polyclonal	+	+	+	+	+	+

* Plug day — day 0.
[‡] Embryo proper.

Immunoreactivity of Fetal and Adult Structures with Monoclonal and Polyclonal Antibodies to Laminin

Antibodies	Reichert's membrane	Visceral yolk sac	Lung*	Kidney	Adrenal	Striated muscle cells	Brain	Liver‡	Stomach and intestine	Skin
14-d fetus										
Monoclonal										
LAM-I	+	−	+	+	−	−	−	−/−	+	+
LAM-II	+	−	+	+	−	−	−	−/−	−	−
Polyclonal	+	+	+	+	+	+	+	+/+	+	+
Adult										
Monoclonal										
LAM-I			+	+	+§	+	ND	−/−	+	+
LAM-II			+	+	+§	−	ND	−/−	−	−
Polyclonal			+	+	+	+	ND	−/+	+	+

* Bronchial basement membranes.
‡ Sinusoids/bile ducts.
§ Only the cortex showed staining.
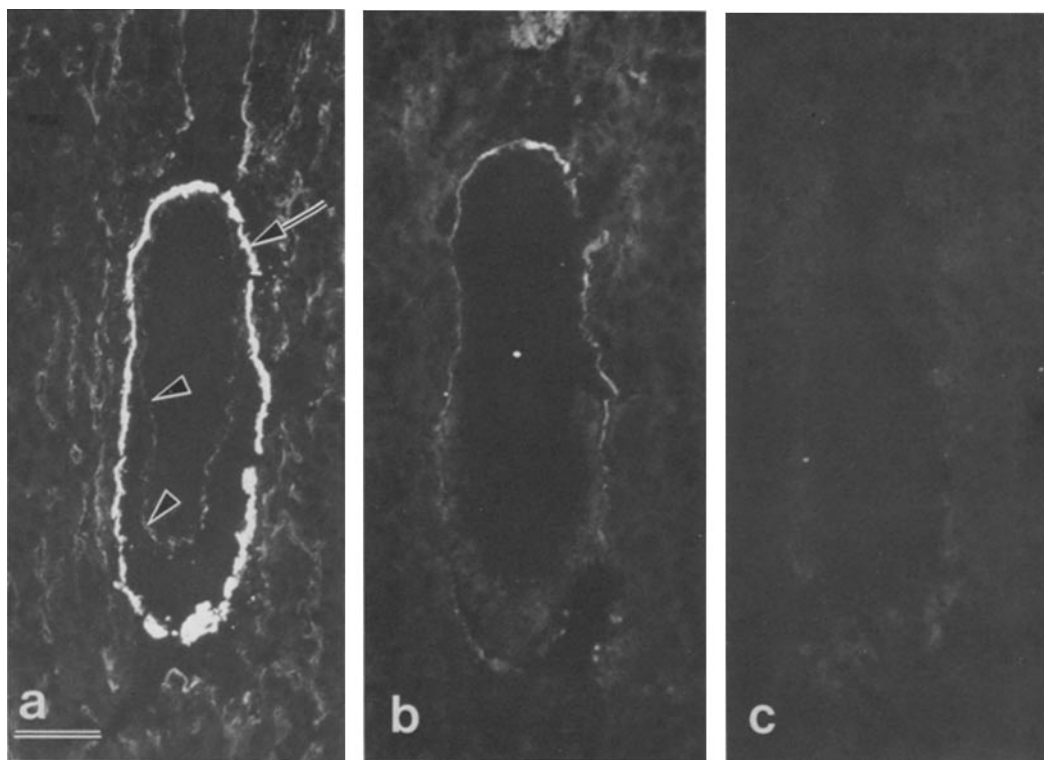
FIGURE 1   6-d mouse egg cylinder. (a) Polyclonal antibody outlines the Reichert's membrane (arrow) and the basement membrane between the visceral endoderm and ectoderm (arrowheads). (b) Monoclonal antibody LAM-I reacts only with Reichert's membrane. (c) Monoclonal antibody LAM-II does not react with the 6-d embryo. Bar, 50 μm. × 160.

1 b) but not with the pericellular matrix of the deciduoma or the embryonic membrane between the visceral endoderm and the ectoderm. The monoclonal antibody LAM-II did not react with any embryonic membrane or the deciduoma (Fig. 1 c). By day 8 of pregnancy, the Reichert's membrane became immunoreactive with all three antibodies and remained strongly immunoreactive throughout the pregnancy (Fig. 2). At this stage the deciduoma also became reactive with both monoclonal antibodies. However, the basement membranes of the embryo proper examined up to day 12 of pregnancy reacted only with the polyclonal antibody and not with the two monoclonal antibodies. The basement membrane underlying the visceral endoderm was also unreactive with the two monoclonal antibodies although strongly reactive with the polyclonal antibodies (Table I). Treatment of sections with pepsin, neuraminidase, chondroitinase, hyaluronidase, or mixed glycosidases before incubation with antibodies did not change the reactivity of the extracellular matrix. In nonfixed sections the background was too high owing to nonspecific binding of the secondary antibody to fetal tissues and membranes which could not be adequately reduced with unlabeled rabbit anti-mouse IgG antiserum used for preincubation. Treatment of sections with mixed glycosidases considerably reduced the background staining, obviating the need for preincubation of sections with the unlabeled rabbit anti-mouse IgG serum.

FIGURE 2   8-d mouse egg cylinder reacted with monoclonal antibody LAM-II. Reichert's membrane reacts strongly, but other basement membranes of the embryo proper are unreactive. The decidual cells are also immunoreactive but weaker than the Reichert's membrane. Bar, 50 μm. × 160.
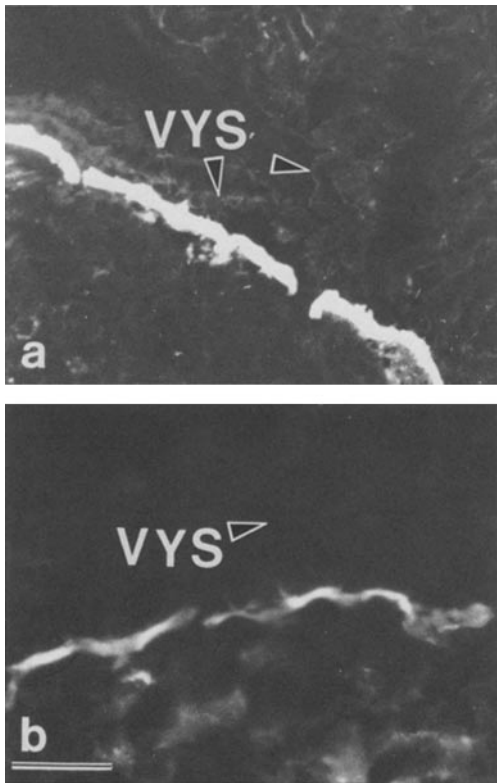

FIGURE 3   Yolk sac of a 14-d mouse fetus. (a) Polyclonal antibody outlines the Reichert's membrane and the membranes underlying the visceral yolk sac (VYS). (b) Monoclonal antibody LAM-I reacts only with the Reichert's membrane. Some reactivity is seen in the adjacent placental tissue in the lower part of the figure. Bar, 50 μm. × 280.

Fetal Stages of Development (14–18 d)

Fetuses examined on day 14 of pregnancy were surrounded by a thick Reichert's membrane strongly reacting with all antibodies (Table II). The basement membrane of the visceral yolk sac was outlined only with the polyclonal antibody (Fig. 3). The fetal skin showed immunoreactivity with the polyclonal antibody and the LAM-I monoclonal antibody, but not with the LAM-II monoclonal antibody (Fig. 4). The polyclonal antibody outlined the contours of the epithelial cells of the entire epidermis and strongly reacted with the basement membrane underlying the epidermis. In the dermis the antibody reacted with the small blood vessels and the smooth muscle cells. The monoclonal antibody LAM-I reacted only with the basement membrane underlying the epidermis, but not with the epidermis or the dermal structures (Fig. 4b).

In the gastrointestinal tract the polyclonal antibodies reacted strongly with the basement membrane separating the epithelium from the stroma. LAM-I monoclonal antibody reacted with the basement membrane although much weaker than did the polyclonal antibody (Fig. 5a). The monoclonal antibody LAM-II was unreactive (Fig. 5b). In other internal organs, the pattern of immunoreactivity varied from one
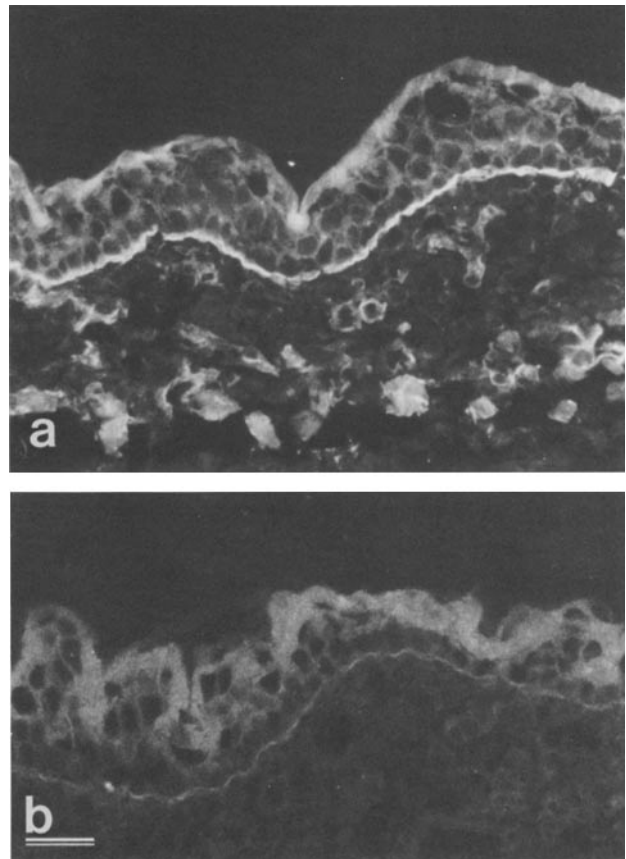

FIGURE 4   Dorsal skin of a 14-d mouse fetus. (a) Polyclonal antibody reacts with the basement membrane at the epidermo-dermal junction and outlines the contours of epidermal cells. The immunofluorescence in the superficial layer of the skin is due to nonspecific binding and was seen in the controls as well. In the dermis the antibody reacts with blood vessels and smooth muscle cells. (b) Monoclonal antibody LAM-I reacts with the epidermal basement membrane but does not react with the epidermal cells. The fluorescence in the upper layer of the epidermis was due to nonspecific binding of the secondary antibody. Bar, 50 μm. × 280.

anatomic site to another. In the lung, the basement membranes of branching bronchi were immunoreactive with all three antibodies (Fig. 6). The basement membranes of the kidney also reacted with all three antibodies. On the other hand, in the brain, liver, adrenal, and in the striated muscles, immunoreactivity was seen only with the polyclonal antibody. In the brain, the polyclonal antibodies outlined the contours of cells both in the developing cortex and the medulla as well as in the blood vessels. In the liver, the reaction was the outlining of the sinusoids and the basement membranes around the branching ductules and blood vessels. In the adrenal, the polyclonal antibodies outlined the sinusoids and the blood vessels in the medulla and the capsule. In the striated muscles, the polyclonal antibodies outlined single cells and reacted with the small and large blood vessels.

The pattern of reactivity observed in the more mature fetus (16, 17, and 18 d) did not change from the pattern seen in the 14-d fetus, with one notable exception. The skin of the 17-d fetus became immunoreactive with the monoclonal antibody (LAM-I (Fig. 7), which reacted with the epidermis in a manner identical to that of the polyclonal antibody. This consisted of reactivity with the basement membrane at the epidermo-dermal junction and reaction with the basal epidermal cell layer and one or two layers of suprabasal cells. The top layers of the epidermis were unreactive. Even at this stage, the dermal structures remained unreactive with the monoclonal antibodies but reacted strongly with the antibody, as in the 14-d fetus. Immunohistochemistry performed on unfixed sections or sections pretreated with various enzymes gave identical results, although as before, the background was

Figure 6   Lung of a 14-d mouse fetus. The basement membranes of the branching bronchi are outlined with the monoclonal antibody LAM-II. The blood vessels are unreactive. Bar, 50 μm. × 160.

Figure 7   Skin of 17-d mouse fetus. Monoclonal antibody LAM-I reacts with the epidermal basement membrane and also outlines the contours of the basal and suprabasal layer of the epidermis. Bar, 50 μm. × 280.
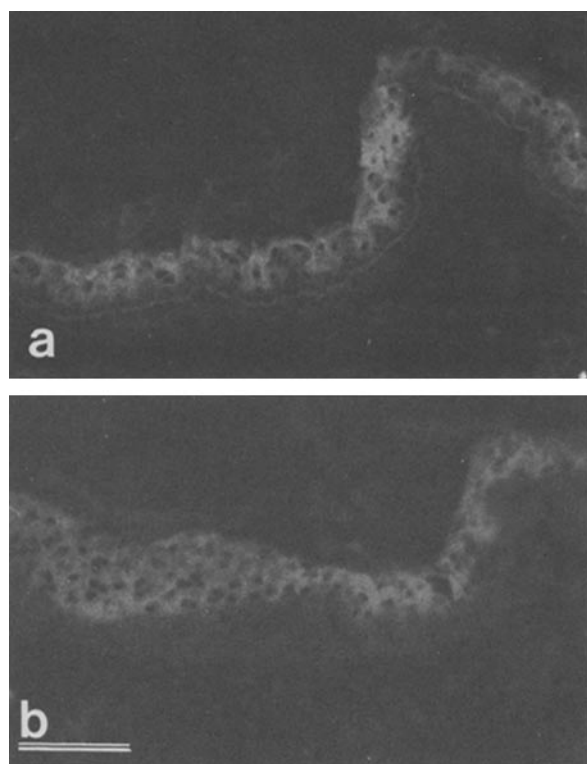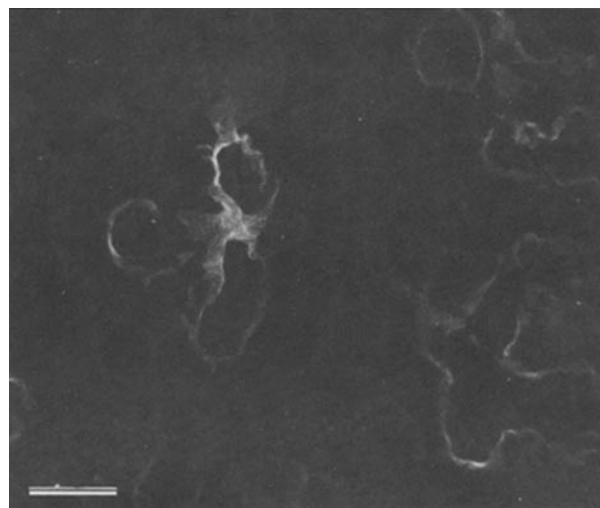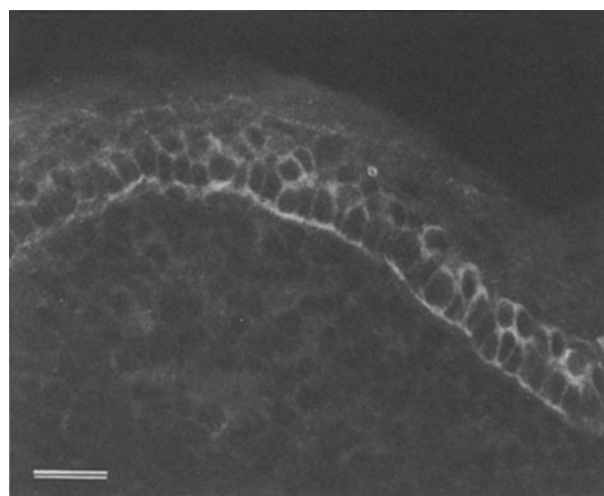

Figure 5   Stomach of a 14-d mouse fetus. (a) Monoclonal antibody LAM-I reacts with the basement membrane underlying the mucosa. The fluorescence of the superficial layers of the mucosa is due to nonspecific binding of the secondary antibody. (b) Monoclonal antibody LAM-II does not react with stomach of the 14-d fetus. Bar, 50 μm. × 280.

considerably higher in the unfixed sections and the treatment with mixed glycosidases markedly reduced the background staining.

Newborn Mouse

The immunoreactivity of various tissues in the newborn mouse did not differ from the same tissues in the fetus, except that the basement membranes of all organs appeared to be thicker. Organs rich in smooth muscle cells such as the gastrointestinal tract reacted more prominently, probably due to an increased size and number of smooth muscle cells.

Adult Mouse

The polyclonal antibodies reacted with the basement membranes of all organs in a pattern similar to the one seen in fetal tissues with two notable exceptions. In the liver, the basement membranes of the blood vessels and the biliary ductules remained immunoreactive, but the immunoreactiv-

ity of the sinusoids disappeared. In the skin, the epidermis became unreactive except for the basement membrane at the epidermo-dermal junction and the basal layer of the epidermis. In the dermis, the polyclonal antibodies reacted with basement membrane of the hair follicles and the smooth muscle cells of errectores pillorum as well as of the blood vessels and nerves (Fig. 8 a). The squamous epithelium of the oral cavity showed reactivity similar to that of the skin (Fig. 9 a). The basement membranes of the striated muscle fibers reacted strongly.

The immunoreactivity of the monoclonal antibodies varied from one tissue to another. In the lung and kidney both monoclonal antibodies reacted with the basement membranes of all structures outlined with the polyclonal antibody. In the skin LAM-I monoclonal antibody reacted with the basement membrane of the epidermis, the hair follicles, and basal cell layer, but not with the smooth muscle cells or nerves (Fig. 8 b). LAM-II monoclonal antibody did not react with adult mouse skin (Fig. 8 c). Similar patterns of reactivity were seen in the oral mucosa, the monoclonal antibody LAM-I reacting with epithelial basement membrane and basal cell layer (Fig. 9 b) and the antibody LAM-II being unreactive. The striated muscle cells were weakly outlined with the LAM-I mono-

clonal antibody and not at all with the LAM-II monoclonal antibody (Fig. 9, b and c). In the adrenal, both monoclonal antibodies reacted exclusively with extracellular matrix surrounding groups of cells in the outer cortex (Fig. 10 a), whereas in slides stained with the polyclonal antibody, the sinusoidal reaction extended throughout the entire cortex (Fig. 10 b). The pretreatment of sections with various enzymes before incubation with the primary antibody did not change the reactivity of tissues with either the polyclonal or monoclonal antibodies.

DISCUSSION

It has been shown that embryonic cells synthesize and secrete basement membrane glycoproteins from the earliest stages of development onward (6–8). However, the extracellular matrix of early mouse embryos differs biochemically from the matrix of later stage embryos, since the embryonic cells do not synthesize and secrete all the major components of the mature adult basement membranes synchronously. Thus, it has been shown that extracellular laminin can be demonstrated as early as the eight-cell stage of development, whereas the first traces of collagen type IV and entactin appear only in the blastocyst
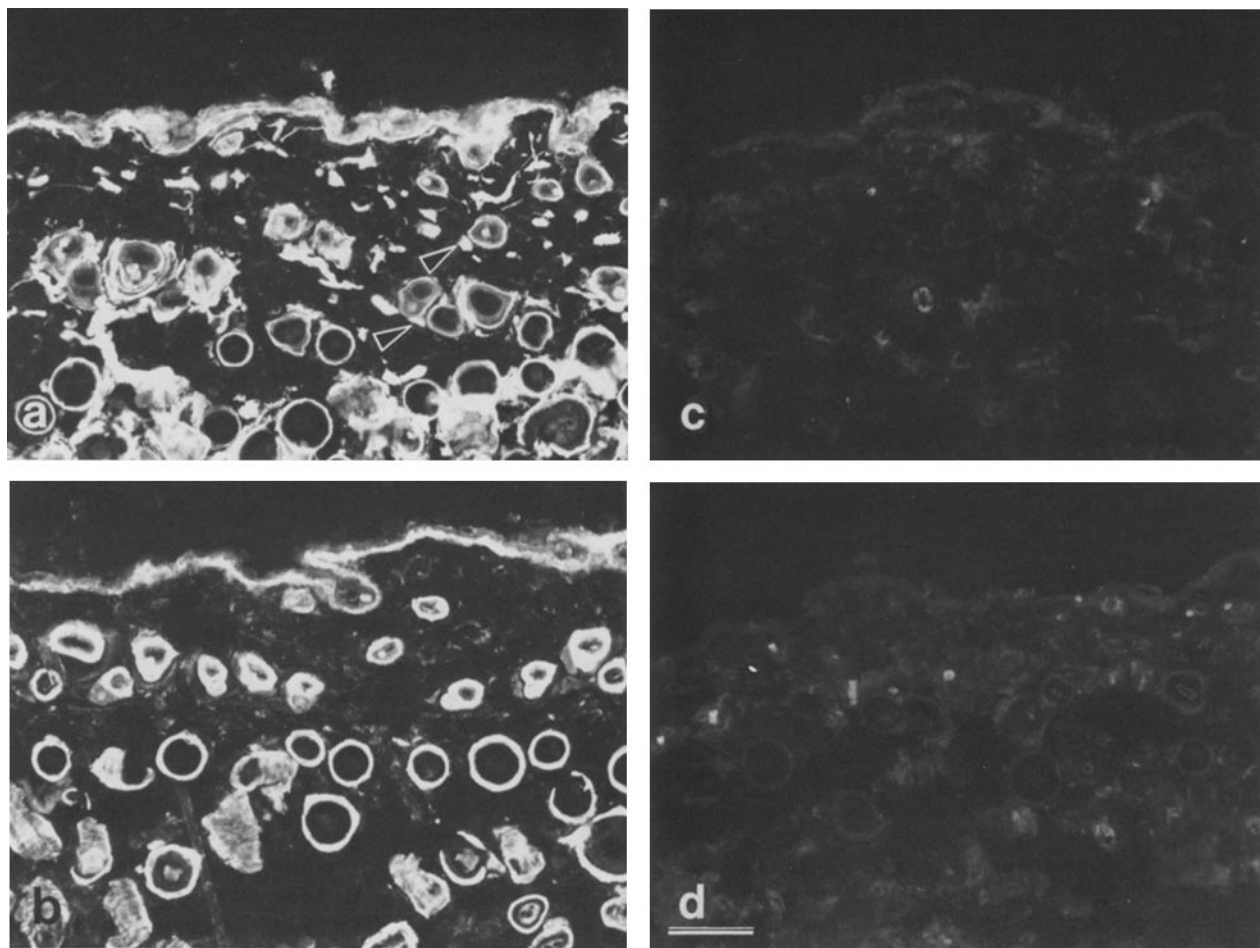

FIGURE 8   Skin of adult mouse. (a) Polyclonal antibody reacts strongly with basement membranes of the epidermis and hair follicles. Also note the reactivity of perifollicular smooth muscle cells of the errectores pillorum (arrowheads). Small blood vessels and nerves, although not distinguishable from one another at this magnification, are seen in the upper dermis. These three structures are not stained with monoclonal antibody LAM-I (compare with b). (b) Monoclonal antibody LAM-I reacts only with the basement membranes of the epidermis and hair follicles. (c) Monoclonal antibody LAM-II does not react with mouse skin. (d) Control. Bar, 50 μm. × 160.
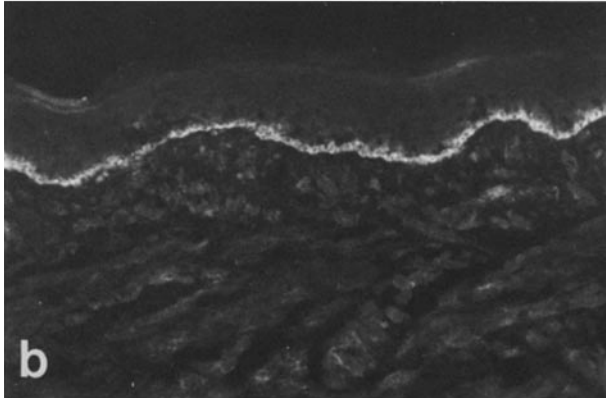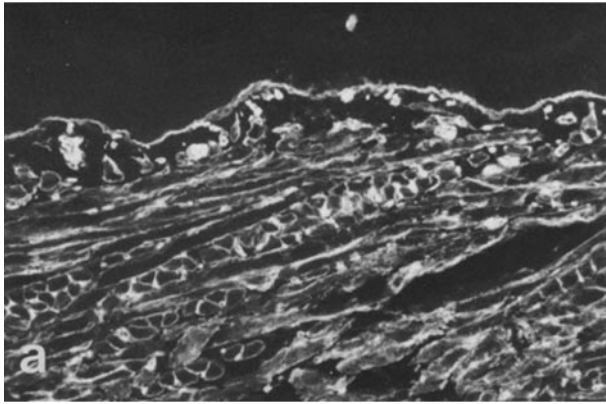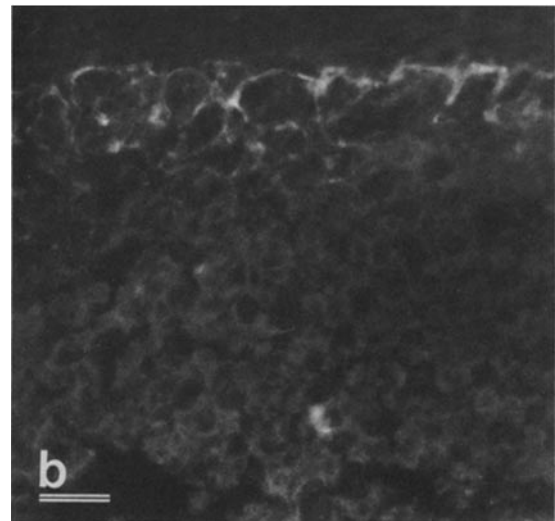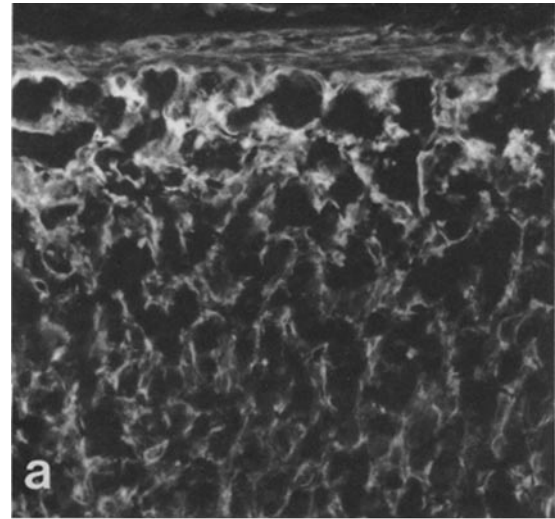
FIGURE 10 Adrenal of an adult mouse. (a) Monoclonal antibody LAM-II reacts with the extracellular matrix surrounding groups of cells in the outer cortex. (b) Polyclonal antibody reacts with the basement membranes of the sinusoids throughout the entire adrenal cortex as well as the matrix surrounding the groups of cells in the outer cortex. Bar, 50 μm. $\times$ 160.

FIGURE 9 Tongue of adult mouse. (a) Polyclonal antibody reacts strongly with the basement membrane underlying the epithelium, the blood vessels, nerves, and the individual striated muscle cells. (b) Monoclonal antibody LAM-I reacts strongly with the basement membrane underlying the epithelium and outlines individual cells of the basal layer of the epithelium. The basement membranes of individual striated muscle cells are weakly reactive. (c) Monoclonal antibody LAM-II does not react with any component of the tongue. Bar, 50 μm. $\times$ 160.

(6, 8). On the other hand, the extracellular matrix from later stages of embryogenesis and the fetal tissues seem to contain all major glycoproteins that are found in the adult organs.

In the present paper we show that laminin of embryonic and extraembryonic basement membranes differs in its immunoreactivity with polyclonal and monoclonal antibodies from that in older embryos and most basement membranes of the adult. This observation could have several possible explanations. First, it is possible that the molecule excreted by the embryonic cells does not contain all the components of the complete laminin molecule. Laminin, a molecule of

M_r 10^6 consists of three subunits, and it is possible that the entire molecule is not assembled in the early embryos. However, the biochemical analysis of early mouse embryos has revealed the presence of all three subunits of laminin in early cleavage stage embryos (7), thus making this explanation unlikely. Second, it is possible that the monoclonal antibodies have a lower affinity for laminin than the polyclonal antibodies. Since there is ostensibly less extracellular matrix in the early embryo, and therefore fewer antigenic sites available, it is quite possible that the monoclonal antibodies did not produce a visible antigen-antibody reaction product. Thirdly, it is quite feasible that the embryonic extracellular matrix contains laminin in a masked form and that it thus cannot be recognized unless the masking substance is removed. However, the treatment of embryonic tissues with several enzymes did not alter the immunoreactivity of laminin-containing extracellular matrix, again suggesting that this explanation is not valid. Furthermore, our immunoprecipitation data (4) show that these antibodies precipitate the unglycosylated molecule derived from tunicamycin-treated endodermal cells and

therefore that the epitope resides in the polypeptide backbone of the antigen. This backbone appears, at least in tissue sections, to be resistant to pepsin digestion. Finally, it is quite feasible that laminin excreted by the early embryonic cells differs sterically or structurally from laminin in older embryos. Our data are compatible with the suggestion that extracellular laminin undergoes conformational or structural changes in the extracellular milieu accounting for its immunoreactivity first with one and then with the second monoclonal antibody. Also, since these antibodies do not immunoprecipitate fragments of laminin <150,000 daltons (4), it is possible that in some tissues and at early stages of development the entire molecule is not fully assembled. However, since we do not know whether these antibodies recognize either sequential or conformational determinants, no definitive interpretation of present findings is possible.

The best example of the differences between the early and late form of extracellular matrix in a defined anatomic structure is Reichert's membrane (12). This basement membrane is the secretory product of the parietal endodermal cells of yolk sac (13–15), a distinct cell layer that is formed in the early postimplantational stages of development and persists to the end of pregnancy. First it is known as distal or parietal endoderm (16) and then expands to form the parietal layer of the yolk sac surrounding the entire mouse embryo. The terms distal endoderm, parietal endoderm, and parietal yolk sac (PYS)[1] have been used interchangeably since all these cells secrete extracellular basement membrane material and are ultrastructurally identical (17–19). The malignant equivalent of the mouse PYS cells has been labeled parietal yolk sac carcinoma (20). Numerous studies have shown a striking similarity between the normal PYS cells and the PYS carcinoma cells (9, 20–22), but it was never explicitly stated whether the PYS carcinoma cells correspond to early parietal endoderm or later stage PYS epithelium. Since the monoclonal antibodies used in this study have been produced by immunizing animals with laminin extracted from PYS cell line derived from a mouse teratocarcinoma (4), and since both of these antibodies react with the extracellular matrix produced by these cells and other mouse and rat PYS carcinomas (I. Damjanov, unpublished observation), one can now, for the first time, state that the PYS carcinoma cells actually correspond more closely to the mid-gestational PYS cells than to the early postimplantational parietal endodermal cells.

The parietal and visceral endoderm, like the later stage parietal and visceral yolk sac cells, secrete laminin-containing basement membranes. The basement membrane of the parietal endoderm is clearly visible by electron microscopy, but the visceral endodermal membrane cannot be seen in ultrastructural preparations (17), although it is easily demonstrable immunohistochemically with antibodies to epithelial basement membrane (23) and to laminin (6, 8). In the present study, we have shown that these two basement membranes also differ with regard to the immunoreactivity with monoclonal antibodies. This coupled with our previous observations (24) that fluoresceinated lectin from Bandeiraea simplicifolia binds to the parietal but not the visceral basement membranes in the early stages of development suggests that these two basement membranes differ one from another. We have not established whether these differences are qualitative or simply reflect the low concentration of laminin in the

_____

[1] *Abbreviation used in this paper:* PYS, parietal yolk sac.

visceral as compared with the high concentration of laminin in the parietal basement membrane.

The visceral yolk sac basement membrane becomes reactive with Bandeiraea simplicifolia in mid-gestation, but the visceral yolk sac basement membrane of the mid-gestation or even late gestation does not become reactive with the monoclonal antibodies to laminin used in the present study. These differences all suggest that certain rearrangements of structural components occur in the basement membranes but that these changes do not necessarily occur in a predictable or uniform manner in all extracellular matrices.

Differential reactivity of embryonic basement membranes with the polyclonal and the two monoclonal antibodies was noted not only in the presomitic embryo but also in several tissues of the fetus and adult mouse. In contrast to the reactivity of the Reichert's membrane, which changed from selectively reactive to fully reactive with both monoclonal antibodies, the basement membranes of stomach and skin of the fetus remained selectively reactive in adult animal as well. On the other hand, striated muscle cells membranes were unreactive with both monoclonal antibodies in the fetus and then became reactive only with the monoclonal antibody LAM-I. Still further, a third pattern was represented by the basement membranes of the kidney and lung which were reactive both in the fetus and the adult with both monoclonal antibodies. Hence, it would appear, at least as revealed immunohistochemically with the two monoclonal antibodies, that certain basement membranes change from "fetal" to "adult" whereas others do not and thus have, even in the fetal tissues, the typical "adult" structure.

In the present study, we have shown that the antibodies to laminin do not react only with the extracellular basement membranes but may also react with some fetal cells. It is not yet clear whether these cells such as fetal skin and liver express laminin-like surface molecules cross-reacting with the antibodies to laminin or whether they secrete laminin. We would be inclined to favor the latter explanation since it has been shown that developing astrocytes (25) and neuroblastoma cells (26) synthesize laminin, although it is not found in the adult central nervous system. It also appears that regenerating liver cells temporarily synthesize laminin (27) in the sinusoidal areas in a pattern resembling the distribution of laminin in fetal mouse liver in the present study. Thus, it would appear that laminin or laminin-like substances are synthesized by cells during development and growth. However, this laminin is not part of a basal lamina and probably has a different role than the laminin deposited in extracellular basement membranes.

REFERENCES

1. Chung, A. E., I. L. Freeman, and J. E. Braginski. 1977. A novel extracellular membrane elaborated by a mouse embryonal-carcinoma-derived cell line. *Biochem. Biophys. Res. Commun.* 79:859–864.
2. Timpl, R., H. Rohde, P. G. Ribey, S. I. Rennard, J.-M. Foidart, and G. R. Martin. 1979. Laminin, a glycoprotein from basement membranes. *J. Biol. Chem.* 254:9933–9937.
3. Martinez-Hernandez, A., and P. S. Amenta. 1983. The basement membrane in pathol-

ogy. *Lab. Invest.* 48:656–677.

4. Chung, A. E., R. Jaffe, B. Bender, M. Lewis, and M. Durkin. 1983. Monoclonal antibodies against the GP-2 subunit of laminin. *Lab. Invest.* 49:576–581.

5. Peters, P. B., and I. J. Goldstein. 1979. The use of fluorescein-conjugated Bandeiraea simplicifolia B4-isolectin as a histochemical reagent for the detection of α-D-galactopyranosyl groups. *Exp. Cell Res.* 120:321–334.

6. Leivo, I., A. Vaheri, R. Timpl, and J. Wartiovaara. 1980. Appearance and distribution of collagens and laminin in the early mouse embryo. *Dev. Biol.* 76:100–114.

7. Cooper, A. R., and H. A. MacQueen. 1983. Subunits of laminin are differentially synthesized in mouse eggs and early embryos. *Dev. Biol.* 96:467–471.

8. Wu, T.-C., Y.-J. Wan, A. E. Chung, and I. Damjanov. 1983. Immunohistochemical localization of entactin and laminin in mouse embryos and fetuses. *Dev. Biol.* 100:496–505.

9. Martinez-Hernandez, A., E. J. Miller, I. Damjanov, and S. Gay. 1982. Laminin-secreting yolk sac carcinoma of the rat. Biochemical and electron immunohistochemical studies. *Lab. Invest.* 47:247–257.

10. Chung, A. E., R. Jaffe, I. L. Freeman, J.-P. Vergnes, J. E. Braginski, and B. Carlin. 1979. Properties of a basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma-derived cell line. *Cell.* 16:277–287.

11. Garvey, J. S., N. E. Cremer, and D. H. Sussdorf. 1981. Methods in Immunology. W. A. Benjamin, New York. Third edition. 313 pp.

12. Pierce, G. B., and P. K. Nakane. 1967. Antigens of epithelial basement membrane of mouse, rat and man. *Lab. Invest.* 17:499–514.

13. Clark, C. C., and N. A. Kefalides. 1982. Partial characterization of collagenous and noncollagenous basement membrane proteins synthesized by the 14.5-d rat embryo perietal yolk sac *in vitro. Connect. Tissue Res.* 10:61–76.

14. Laurie, G. W., C. P. Leblond, G. R. Martin, and M. H. Silver. 1982. Intracellular localization of basement membrane precursors in the endodermal cells of the rat perietal yolk sac. III. Immunostaining for laminin and its precursors. *J. Histochem. Cytochem.* 30:991–997.

15. Semoff, S., B. L. M. Hogan, and C. R. Hopkins. 1982. Localization of fibronectin,

laminin-entactin and entactin in Reichert's membrane by immunoelectron microscopy. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1171–1175.

16. Gardner, R. L. 1982. Investigation of cell lineage and differentiation in the extraembryonic endoderm of the mouse embryo. *J. Embryol. Exp. Morphol.* 68:175–198.

17. Solter, D., I. Damjanov, and N. Skreb. 1971. Ultrastructure of mouse egg-cylinder. *Z. Anat. Entwicklungsgesch.* 132:291–298.

18. Adamson, E. D., and S. E. Ayers. 1979. The localization and synthesis of some collagen types in developing mouse embryos. *Cell.* 16:953–965.

19. Martinez-Hernandez, A., P. K. Nakane, and G. B. Pierce. 1974. Intracellular localization of basement membrane antigen in parietal yolk sac cells. *Am. J. Pathol.* 76:549–555.

20. Pierce, G. B. Jr., A. R. Midgley, Jr., J. Sri Ram, and J. D. Feldman. 1962. Parietal yolk sac carcinoma: clue to the histogenesis of Reichert's membrane of the mouse embryo. *Am. J. Pathol.* 41:549–566.

21. Pierce, G. B., A. Jones, N. G. Orfanakis, P. K. Nakane, and L. Lustig. 1982. Biosynthesis of basement membrane by parietal yolk sac cells. *Differentiation.* 23:60–72.

22. Leivo, I. 1983. Basement membrane-like matrix of teratocarcinoma-derived endodermal cells: presence of laminin and heparan sulfate in the matrix at points of attachment to cells. *J. Histochem. Cytochem.* 31:35–45.

23. Pierce, G. B. 1966. The development of basement membrane of mouse embryo. *Dev. Biol.* 13:231–249.

24. Wu, T.-C., Y.-J. Wan, and I. Damjanov. 1983. Fluorescein-conjugated Bandeiraea simplicifolia lectin as a marker of endodermal, yolk sac, and trophoblastic differentiation in the mouse embryo. *Differentiation.* 24:55–59.

25. Liesi, P., D. Dahl, and A. Vaheri. 1983. Laminin is produced by early rat astrocytes in primary culture. *J. Cell. Biol.* 96:920–924.

26. Liesi, P. 1983. Laminin in cultured mouse C 1300 neuroblastoma cells: immunocytochemical localization by pre and postembedding electron microscope procedures. *J. Histochem. Cytochem.* 31:755–764.

27. Carlsson, R., E. Engvall, A. Freeman, and E. Ruoslahti. 1981. Laminin and fibronectin in cell adhesion: enhanced adhesion of cells from regenerating liver to laminin. *Proc. Natl. Acad. Sci. USA* 78:2403–2406.