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Epithelial Basement Membrane of Mouse Jejunum
Evidence for Laminin Turnover along the Entire Crypt–Villus Axis

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Abstract
Little is known regarding turnover of the epithelial basement membrane in adult small intestine. Are components degraded and inserted along the length of the crypt–villus axis or selectively in the crypt region with subsequent migration of basement membrane from crypt to villus tip in concert with epithelium? We injected affinity-purified sheep anti-laminin IgG or sheep anti-laminin IgG complexed to horseradish peroxidase (HRP) into mice to label basement membrane laminin in vivo. Fluorescence microscopy revealed linear fluorescence along the length of the jejunal epithelial basement membrane 1 d after anti-laminin IgG injection. By 1 wk, small nonfluorescent domains were interposed between larger fluorescent domains. Over the next 5 wk the lengths of nonfluorescent domains increased progressively whereas those of fluorescent domains decreased. Additionally, electron microscopy revealed HRP reaction product along the length of the epithelial basement membrane after 1 d whereas unlabeled or lightly labeled domains that increased in length with time were observed interposed between heavily labeled domains by 2 and 4 wk along the entire crypt–villus axis. We conclude that laminin turnover occurs focally in the epithelial basement membrane of mouse jejunum along the crypt–villus axis over a period of weeks and that this basement membrane does not comigrate in concert with its overlying epithelium. (J. Clin. Invest. 1990. 86:87–95.) Key words: extracellular matrix • immunocytochemistry • immunofluorescence • morphometry • small intestine

Introduction
Polarized epithelia are intimately associated with extracellular matrix components that are organized into a basement membrane, which underlies their basal surface (1, 2). Epithelial basement membranes provide structural support for the epithelium to which they are applied. In addition, there is substantial evidence that interactions between epithelial cells and the extracellular matrix, particularly basement membrane compo-

nents (a) promote cell adhesion to the extracellular matrix (3, 4), (b) induce expression of differentiated epithelial cell functions (5, 6), (c) initiate and maintain cell polarity (7, 8), (d) participate in the regulation of morphogenesis (9, 10), and (e) promote cell migration (11). In mature adult organs with limited cell proliferation, basement membranes are generally quite stable structures (12, 13). In tissues that proliferate actively during morphogenesis, in contrast, there is modification and more rapid turnover of basement membrane components (12, 13).

The basement membrane underlying the epithelium of the small intestine is a thin sheet 50–75 nm wide with scattered round and oval defects (14, 15). These gaps may be related to the trafficking of leukocytes, especially lymphocytes, between the lamina propria and the epithelial intercellular spaces and to the projection of pseudopod-like processes of epithelial cell basal cytoplasm into the lamina propria where they contact mesenchymal cells (14–16). The major components of the epithelial basement membrane of the small intestine, like that of other epithelial basement membranes, include type IV collagen, laminin, entactin/nidogen, and heparan sulfate proteoglycan in addition to the interstitial matrix components, fibronection and type III procollagen (2, 17).

Unlike many epithelial organs in the adult, those of the tubular alimentary tract are characterized by rapid cell renewal of the epithelium. In the mammalian small intestine active epithelial cell proliferation is confined to crypts. Cells differentiate as they migrate out of the crypts and up the villi to the villus tips from which they exfoliate within 3–6 d after their formation in crypts (14). The small intestine would therefore appear to provide a useful model in which to examine in vivo the turnover of the components of a basement membrane applied to an epithelium composed of proliferating, differentiating, and mature cell populations.

In the small intestine, a sheath of pericryptal myofibroblasts is apposed to the abluminal surface of the basement membrane (18–20). Evidence for proliferation by pericryptal fibroblasts with their subsequent migration toward the villus tip led to the speculation that epithelium, basement membrane, and mesenchyme may normally migrate synchronously as a unit from crypt to villus tip (18). If true, progressive replacement of basement membrane from mid-crypt to villus tip would be expected in 3–6 d with each migration cycle. However, in other studies coordinated migration of fibroblasts and epithelium in small intestine could not be demonstrated (21, 22).

It has recently been shown that intravenous injection of polyclonal anti-laminin IgG into mice and rats labels basement membranes of organs with fenestrated capillaries, including those of the mucosa of the small intestine (23, 24). In the studies reported here, we labeled basement membrane of
the intestinal mucosa of mice in vivo with anti-laminin IgG and anti-laminin IgG coupled directly to horseradish peroxidase (HRP). We used immunofluorescence and electron microscopy to assess the location of labeling and its subsequent decay along the crypt–villus axis and to determine whether or not the basement membrane migrates in concert with its overlying epithelium.

**Methods**

**Animals.** Male, adult BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 22–26 g were used in these studies.

**Proteins and reagents.** Laminin from murine Englebreth-Holm-Swarm tumor was purified (25, 26). The presence of other extracellular matrix components in this laminin preparation, including fibronectin, proteoglycans, or entactin/nidogen was not detected immunochromatically (25), on polyacrylamide gels (24, 25, 27) or by rotary shadow electron microscopy (27). Anti-laminin IgG from previously immunized sheep was affinity-purified as described earlier (24, 25, 28) using laminin-Sepharose columns and was highly specific for laminin with no cross-reactivity with type IV collagen by an enzyme-labeled immunosorbent assay (28) or by Western blotting (data not shown).

Sheep anti-laminin IgG and, for control studies, chromatographically pure sheep IgG (Organon Teknika-Cappel Laboratories, West Chester, PA) were conjugated directly (29) to activated HRP (type VI, Sigma Chemical Co., St. Louis, MO) as before (24, 25, 28). Rhodamine-conjugated rabbit anti-sheep IgG was obtained from Organon Teknika-Cappel Laboratories.

**Administration of sheep anti-laminin IgG to mice.** Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (0.75–1 mg). The saphenous vein was exposed and 1 mg of anti-laminin IgG or control IgG in 0.3–0.5 ml of PBS (pH 7.4) was injected using a 30-gauge needle. Additional mice received 0.5 mg of anti-laminin IgG-HRP or, as controls, sheep IgG-HRP in 0.5 ml of PBS intravenously. The skin was sutured with silk and the mice were permitted to resume feeding and awareness upon awakening. Two mice were studied for each time point examined with each of the experimental IgG preparations.

**Immunofluorescence microscopy.** Mice were fasted overnight in metabolic cages but allowed access to water. Under sodium pentobarbital anesthesia the abdomen was opened via a midline incision and segments of proximal jejunum were removed 1, 7, 24, and 42 d after injection of anti-laminin IgG and 1 d after injection of control IgG. Unfixed pieces ~5 mm² were placed on cryostat chucks in OCT embedding medium (Miles Laboratories, Elkhart, IN) and frozen in isopentane cooled in liquid nitrogen. Cryostat sections, 4 µm thick, were mounted on poly-L-lysine-coated coverslips, air dried, and fixed for 5 min in acetone. After rehydration in PBS containing 0.02% gelatin, sections were incubated with rhodamine-conjugated rabbit anti-sheep IgG for 60 min at room temperature and mounted in glycerol containing 0.1% p-phenylenediamine. Additional sections from mice killed 42 d after injection were preincubated with sheep anti-laminin IgG (150 µg/ml) before incubation with rhodamine-conjugated rabbit anti-sheep IgG. Sections were viewed and photographed with a Zeiss microscope (Carl Zeiss, Inc., Thornwood, NY) as described previously (30).

**Immunoperoxidase microscopy.** Segments of proximal jejunum from mice were removed as described above 1, 2, 6, and 4 wk after injection of sheep anti-laminin IgG-HRP and 1 d after injection of sheep control IgG-HRP, and quickly immersed in 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.4) containing 0.01 M CaCl₂ for 2 h. After washing in cacodylate buffer, jejunal segments were embedded in 5% agar and cut into 50-µm sections parallel to the plane of the villi with a vibratome (EBTEC, Aga-

1. Abbreviation used in this paper: HRP, horseradish peroxidase.

Figure 1. Fluorescent antibody staining of frozen sections of mouse jejunal villi. (a) 1 d after injection of sheep anti-laminin IgG followed by in vitro exposure to rhodamine-conjugated rabbit anti-sheep IgG, there is linear fluorescence of the basement membrane underlying the epithelium (E). Fluorescence in the lamina propria (L) in this micrograph and in b, c, and d represents capillary, nerve, and muscle basement membrane. ×1,800. (b) 1 wk after injection of sheep anti-laminin IgG followed by in vitro exposure to rhodamine-conjugated rabbit anti-sheep IgG, the fluorescence of the basement membrane underlying the epithelium (E) has a speckled appearance. ×1,800. (c) 6 wk after injection of sheep anti-laminin IgG followed by in vitro exposure to rhodamine-conjugated rabbit anti-sheep IgG, the segments of basement membrane underlying epithelium (E) with intense fluorescence have decreased in length whereas segments with no or faint fluorescence (arrows) have increased in length. ×1,800. (d) Adjacent section from the villus shown in c preincubated in sheep anti-laminin in vitro before exposure to rhodamine-conjugated rabbit anti-sheep IgG. There is linear fluorescence of the basement membrane underlying the epithelium (E). ×1,800. Photographic exposure for a, b, and c was 15 s whereas exposure for d was 4 s.

Localization of sheep anti-laminin IgG-HRP in jejunal mucosa by electron microscopy. 1 d after injection of anti-laminin IgG-HRP, electron-dense peroxidase reaction product decorated the epithelial basement membrane at the interface of epithelium and the lamina propria along the length of the jejunal villi (Fig. 3 a) and along most of the laminin propria–epithelial interface along jejunal crypts (Fig. 4 a). Gaps in basement membrane labeling were consistently observed.
however, at sites where there was migration of lamina propria cells into the intercellular spaces between epithelial cells (Fig. 5). Gaps in labeling were also seen where processes of epithelial cell cytoplasm projected into the lamina propria as well as in occasional focal areas within intact basement membrane, especially along crypts. Capillary, nerve, and smooth muscle basement membranes in the lamina propria were usually decorated linearly along their length with electron-dense reaction product. In contrast, in mice killed 1 d after injection with control sheep IgG-HRP, reaction product was apparent only in structures with endogenous peroxidase activity (such as peroxisomes) but not along basement membranes.

2 wk after injection of anti-lamin IgG-HRP, focal lengths of basement membrane that were heavily decorated

Figure 2. Fluorescent antibody staining of frozen sections of mouse jejunal crypts. The experimental procedure for a, b, c, and d correspond to a, b, c, and d in Fig. 1. (a) 1 d after injection of sheep anti-lamin IgG, basement membrane fluorescence underlying epithelium (E) is essentially linear with few gaps. Fluorescence in the lamina propria (L) in this micrograph and in b, c, and d represents capillary, nerve, and muscle basement membrane. ∗×1,800. (b) 1 wk after injection of sheep anti-lamin IgG, basement membrane fluorescence underlying epithelium (E) has a speckled pattern. ∗×1,800. (c) 6 wk after injection of sheep anti-lamin IgG, segments of basement membrane with faint or no fluorescence underlying epithelium (E) have increased in length. ∗×1,800. (d) After preincubation in sheep anti-lamin, in vitro fluorescence of a section adjacent to that shown in c demonstrates linear fluorescence of the basement membrane underlying epithelium (E). ∗×1,800.
Figure 3. Electron micrographs of unstained sections at the interface of epithelium and lamina propria from the midvillus region of jejunum from mice that had received sheep anti-laminin IgG-HRP intravenously. (a) 1 d after injection of anti-laminin IgG-HRP, there is HRP-reaction product along the length of the epithelial basement membrane (solid arrows). E, epithelium; L, lamina propria. ×10,400. (b) 2 wk after injection of anti-laminin IgG-HRP, segments of epithelial basement membrane without HRP-reaction product (open arrows) are interposed between segments with reaction product (solid arrows). E, epithelium; I, intraepithelial lymphocyte; L, lamina propria. ×10,400. (c) 4 wk after injection of anti-laminin IgG-HRP, the segments of epithelial basement membrane with no HRP-reaction product have increased in length (open arrows) whereas those with reaction product (solid arrows) have decreased in length. E, epithelium; L, lamina propria. ×10,400.

Figure 4. Electron micrographs of unstained sections of the interface at epithelium and lamina propria from the crypt region of jejunum from mice that had received sheep anti-laminin IgG-HRP intravenously. (a) 1 d after injection of anti-laminin IgG-HRP, there is decoration with HRP-reaction product along the length of the epithelial basement membrane (solid arrows). E, epithelium; L, lamina propria. ×10,400. (b) 2 wk after injection of anti-laminin IgG-HRP, segments of epithelial basement membrane without HRP-reaction product (open arrows) are interposed between segments with reaction product (solid arrows). E, epithelium; L, lamina propria. ×10,400. (c) 4 wk after injection of anti-laminin IgG-HRP, the segments of epithelial basement membrane with faint or no reaction product are longer (open arrows) whereas those with intense reaction product are shorter (solid arrows) than were observed at 2 wk. E, epithelium; L, lamina propria. ×10,400.

with reaction product were interrupted by weakly decorated or unlabeled lengths along villus epithelium (Fig. 3 b) and along crypt epithelium (Fig. 4 b). By 4 wk the domains of epithelial basement membrane with peroxidase activity were smaller and those without activity were larger than was noted at 2 wk both in villi (Fig. 3 c) and in crypts (Fig. 4 c). In contrast, the
basement membrane associated with most nerve fibers and smooth muscle cells but not capillaries remained intensely decorated with reaction product 4 wk after anti-laminin IgG-HRP injection, even when located proximate to sparsely labeled epithelial basement membrane (Fig. 6).

**Morphometry.** Morphometric assessment of labeling of the villus and crypt epithelial basement membrane 1 d, 2 wk, and 4 wk after administration of anti-laminin IgG-HRP is summarized in Fig. 7. The extent of labeling of villus basement membrane decreased from 95% at 1 d to 54% at 2 wk, and to 26% at 4 wk. All three mean values differed from one another (P < 0.01) despite considerable variation among some pairs. Similarly, labeling of crypt basement membranes decreased from 86% at one day to 35% at 2 wk and to 11% at 4 wk. Again, all three mean values differed from one another (P < 0.01).

**Discussion**

These studies, in which laminin was labeled in vivo with anti-laminin IgG, demonstrate that laminin is present throughout the basement membrane that underlies the jejunal epithelium of adult mice from crypt base to villus tip, confirming earlier observations in mice and other mammalian species (17, 24, 33). Moreover, the persistence of some anti-laminin IgG along the length of the crypt-villus axis for as long as 6 wk provides strong evidence that the basement membrane does not comi-

**Figure 5.** Electron micrograph of the epithelial–mesenchymal interface of a jejunal villus from a mouse that had received sheep anti-laminin IgG-HRP intravenously 1 d earlier. There is a gap in the epithelial basement membrane where a lymphocyte (L) is traversing the interface between the epithelium (E) and the lamina propria (L). On each side of the gap the basement membrane is decorated with HRP-reaction product (arrows). Lightly stained with uranyl acetate and lead citrate. ×8,300.

**Figure 6.** Unstained electron micrograph of nerve fibers in lamina propria that are in close proximity to epithelium (E) from a mouse that had received sheep anti-laminin IgG-HRP intravenously 4 wk earlier. The basement membranes enclosing nerve fibers are decorated along most of their length (solid arrows) with HRP-reaction product whereas the adjacent epithelial basement membrane (open arrows) is largely devoid of reaction product. ×12,100.

**Figure 7.** Percentage of jejunal villus and crypt epithelial basement membrane decorated with HRP-reaction product as determined by morphometric measurements from mice killed 1 d, 2 wk, and 4 wk after intravenous injection of sheep anti-laminin IgG-HRP. Each bar shows the mean and SD of measurements from 10 micrographs from each of two mice for the villus basement membrane and the mean and SD of measurements from five micrographs from each of two mice for the crypt basement membrane.
grate with its overlying epithelium or underlying myofibroblasts. Mouse jejunal epithelial cells migrate from their origin in the crypts to the extrusion zone on the tips of villi in 48–72 h (21). Therefore, if comigration of basement membrane and epithelium had occurred, loss of basement membrane labeling in the upper crypt region and base of villi by 1 d and virtual disappearance of basement membrane labeling by 1 wk after anti-laminin IgG injection should have been observed. Instead, our findings indicate that the intestinal epithelial base-

membrane appears to be a relatively stable structure that provides a scaffold on which epithelial cells and perhaps sub-

epithelial myofibroblasts migrate. Additional evidence for mi-
gation of intestinal epithelial cells along their underlying base-

membrane is found in recent studies in which viable epithelial cells rapidly covered denuded basement membrane after destruction of the epithelium on the upper portion of villi (34, 35).

Basement membranes in most tissues of adult animals appear to be remarkably stable structures with a long half-life and slow turnover of their components (13, 36). However, the ep-

ithelial basement membrane of the small intestine and other tubular digestive organs are unusual among those of adult tissues in that they support and interact with cell populations undergoing rapid renewal (14, 37). Hence, substantial turn-

over of basement membrane components might be expected since there is rapid basement membrane assembly in actively growing fetal and neonatal tissues (13, 38) and rapid basement membrane degradation accompanies involution of the post partum mammary gland (39). Although our studies indicate that the overall turnover of the jejunal epithelial basement membrane is slow, they suggest that constant remodeling of the epithelial basement membrane of adult mouse jejunum takes place. When anti-laminin IgG was injected intrave-
nously, the antibodies initially labeled in a linear fashion vir-
tually the entire epithelial basement membrane as assessed by fluorescence and electron microscopy. Subsequently, IgG la-

beling of the basement membrane decreased progressively with the passage of time along the entire crypt–villus axis but the intensity of labeling did not decrease uniformly. Rather, strongly labeled domains were interposed between unlabeled and lightly labeled domains for up to 4 and 6 wk after injection of anti-laminin IgG-HRP (Figs. 3 c and 4 c) and anti-laminin

IgG (Figs. 1 c and 2 c), respectively. However, that the unla-

beled domains contained abundant laminin was shown by in vitro exposure of these tissues to anti-laminin IgG (Figs. 3 d and 4 d). This pattern of fluorescence decay with time in the epithelial basement membrane of adult mouse jejunum dif-

fered strikingly from that observed in glomerular basement membrane of adult rat kidney in which fluorescence remained linear for at least 10 wk after anti-laminin IgG injection (25). On the other hand, the appearance with time of alternating labeled and unlabeled segments along the epithelial basement membrane of the adult mouse jejunum is remarkably similar to that observed in glomerular, adrenal, and pituitary base-

membranes of newborn rats after injection of anti-lami-

nin IgG (13, 28, 38). In those studies, reexpression to anti-lami-
nin IgG in vivo at later times resulted in decoration of the unlabeled basement membrane segments with the antibody leading to the interpretation that newly synthesized matrix is progressively spliced into old in these rapidly growing tissues (12, 28, 38). A similar process may occur during epithelial basement membrane renewal in the jejunum of adult mice.

There are several factors that may contribute to the turn-

over of laminin (and perhaps other matrix components) in the epithelial basement membrane of the mouse intestine. First, local protease production by epithelial or lamina propria cells may regulate, by proteolysis of laminin and other matrix com-

ponents (40), detachment and reattachment of the epithelial cell basal membrane to the basement membrane during crypt to villus migration. If this occurs, however, the laminin in some basement membrane domains appears to be more sus-

cceptible to digestion than in others. Secondly, the movement of leukocytes across the basement membrane as they migrate between the lamina propria and epithelial cell intercellular spaces may have contributed to the discontinuous pattern of basement membrane labeling observed several weeks after anti-laminin IgG injection. Such trafficking of cells from lam-

ina propria to epithelium is a dynamic process (41, 42) and occurs through discontinuities in the basement membrane (Fig. 5). It seems possible that such gaps are produced by the migrating cells at the point of basement membrane penetra-
tion and are then rapidly sealed by the reassembly of matrix components, since basement membrane defects are not usually observed underlying intraepithelial lymphocytes not in the process of traversing the basement membrane. Thirdly, the formation of pseudopod-like processes of epithelial basal cyto-

plasm that extend into the underlying mesenchyme through gaps in the basement membrane (14, 16) may have contrib-

uted to focal basement membrane degradation and its sub-

sequent reassembly. Fourthly, some nonspecific dissociation of bound anti-laminin IgG from its binding sites may have oc-
curred. If so, the turnover of laminin in the epithelial basement membrane of adult mouse jejunum would be even slower than our results indicate. However, the focal loss of anti-laminin

IgG from some basement membrane domains and its reten-

tion in adjacent domains cannot be readily explained by non-
specific dissociation alone. Additionally, the persistence of anti-laminin IgG tracer in basement membranes of nerve fibers juxtaposed to unlabeled epithelial basement membrane (Fig. 6) suggests that mechanisms other than nonspecific disso-

ciation of anti-laminin IgG from laminin contributed to the loss with time of label from the epithelial basement mem-

brane.

Studies in which explants of intestinal endoderm or mesen-

chyme from fetal rats or mice were cultured separately or while in direct contact with one another suggest that the presence of both mesenchyme and epithelium is essential for basement membrane formation and epithelial cell differentiation (43–45). By the use of species specific antibodies and interspes-
cies grafts of rat and chick fetal intestinal mesenchyme and endoderm, it has recently been shown that type IV collagen is produced by mesenchymal cells and heparan sulfate proteo-
glycan is produced by the epithelium in the basement mem-

brane that forms at the epithelial–mesenchymal interface of these grafts (45, 46). In another recent study, expression of laminin mRNA has been detected by in situ hybridization in the lamina propria but not in epithelium of the small intestine of mouse fetuses (47). Less is known regarding the site of pro-
duction of specific basement membrane components in the intestine of adult animals. Expression of laminin mRNA was not detected by in situ hybridization in adult mouse intestine (47) but a preliminary report detected in dot blot preparations type IV collagen α-chain mRNA in the lamina propria and laminin B-chain mRNA in the lamina propria and crypt epi-
thelium of small intestine from adult rats (48). Our studies do not provide information on the cellular origin of new laminin in adult intestine but disappearance of bound anti-laminin IgG3 took place in epithelial basement membrane along the entire crypt–villus axis. This suggests that considerable degradation and assembly of basement membrane underlying both the undifferentiated, differentiating, and differentiated compartments of the epithelium occur in the small intestine of adult mice. Since studies in the small intestine of the adult rat suggest that other basement membrane components, including entactin/nidogen, type IV collagen, and heparan sulfate proteoglycan, like laminin, are distributed uniformly along the crypt–villus axis in the epithelial basement membrane (17), study of the in vivo turnover of these molecules in intestinal mucosa should also be of interest.

In summary, the major findings of this study are (a) laminin turnover occurs focally in the epithelial basement membrane of mouse jejunum along the entire crypt–villus axis over a period of weeks, and (b) the jejunal epithelial basement membrane does not comigrate in concert with its overlying epithelium since the villus epithelium, unlike its basement membrane, is replaced in 2–3 d.

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References


