Basal Lamina Formation on Thyroid Epithelia in Separated Follicles in Suspension Culture

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ABSTRACT When thyroid follicles are isolated by collagenase treatment of minced thyroid lobes, the basal lamina around each follicle is removed. The basal lamina does not reform when follicles are cultured in suspension in Coon's modified Ham's F-12 medium containing, in addition, 0.5% calf serum, insulin, transferrin, and thyrotropin. We have added acid soluble collagen and/or laminin to see if they would result in the formation of a basal lamina. An extended basal lamina did not form when follicles were embedded in a gel formed from acid-soluble rat tendon collagen or from calf skin collagen when added at a concentration of 100 μg collagen/ml. However, laminin at a concentration of 5.1 μg/ml gave rise to short segments of a basal lamina within 30 min. At longer time intervals, the segments lengthened and covered the base of many cells, and were continuous across the gap between cells and across the mouth of a coated pit. Not all basal surfaces were covered, and no exposed apical surfaces with microvilli had a basal lamina. There was no obvious difference in the appearance of the basal lamina if collagen was added in addition to laminin, but collagen, in contact with the plasma membrane when added alone, was lifted off the membrane in the presence of the basal lamina. The basal lamina appeared denser if formed in the presence of 5% serum instead of 0.5%.

It has been possible to maintain small clusters of thyroid epithelial cells in suspension culture (16). The clusters are varied in morphology, many in the form of single follicles, but some contain several microlumens. In 0.5% calf serum the epithelial cells in these follicles maintain normal polarity with a surface covered with microvilli in contact with a lumen filled with an electron-dense colloid. Occasionally there is an additional cell not in contact with the lumen but attached to the follicle wall with a surface containing microvilli in contact with the medium.

The clusters differ from thyroid follicles in vivo in one obvious respect: there is no basal lamina. The basal lamina is removed from the follicles at the time minced thyroid lobes are treated with collagenase to prepare separated follicles, and the basal lamina does not reform even after a week or more in suspension culture.

We have now found that if a low concentration of laminin, a constituent of the basal lamina (8, 14), is added to the medium, a basal lamina is deposited. (See Hay [11] for a recent review of extracellular matrix.) In the present paper we describe the conditions under which a basal lamina is formed and some of its characteristics.

MATERIALS AND METHODS

Primary suspension cultures of thyroid follicles were prepared by collagenase treatment of minced thyroid glands of 6-wk-old male Fischer rats and differential filtration as previously described (16). Newly isolated clusters of thyroid epithelial cells were cultured without shaking in 35-mm agarose-coated tissue culture dishes (Costar, Data Packaging Corp., Cambridge, MA) at 37°C with 95% air and 5% CO2 as gas phase and allowed to form follicles for 1 d. The culture medium was Coon's modified Ham's F-12 containing 0.5% calf serum (donor serum; Flow Laboratories, Inc., Rockville, MD). The medium was changed at the end of one day and was supplemented with 0.1 mIU/ml thyrotropin (3.5 U/mg, bovine thyrotropin, National Hormone and Pituitary Agency, Baltimore, MD), 5 μg/ml human transferrin (Sigma Chemical Co., St. Louis, MO; cat. no. 1-2252), 50 μg/ml ascorbic acid (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA) and 5 ng/ml bovine insulin (Sigma Chemical Co.; cat. no. 1-5500). The insulin concentration used was slightly higher than physiological (5, 10). The high concentrations of insulin ordinarily used in culture media (3) are because of insulin's growth-promoting properties and are unnecessary in our maintenance cultures. When longer term cultures were necessary medium was changed every third day; where indicated the serum level was changed to 5%. Collagen and/or laminin were added to the medium to see if they would influence the formation of a basal lamina.

Collagen

Three samples of collagen were tested. Acid-soluble calf skin collagen (Calbiochem-Behring Corp.; cat. no. 234112) was dissolved in cold 0.1 M acetic acid. Almost all studies were done with purified acid-soluble calf skin collagen and rat tendon collagen (kindly donated by Dr. Hynda Kleinman, National Institute of Dental Research [NIDR]). They were dissolved in cold 0.5 M acetic acid. The collagen solutions were dialyzed in the cold for 6-8 h against the tissue culture medium. Immediately after dialysis the cold dialyzed collagen solution was added to the cultures in the form of small drops to a final concentration of 50 to 100 μg collagen/ml and dishes were gently shaken to distribute the collagen throughout the medium.
Laminin

Purified laminin, stock solution (0.68 mg/ml in Tris buffer, kindly donated by Dr. Hynda Kleinman, NIDR), was used in the culture medium at a concentration of 5.1 µg/ml. Laminin, alone, was added to the cultures on the second day of culture. When studied in combination with collagen it was added at this time and collagen was added a few hours later. If collagen was added first the laminin was usually added 1 d after the collagen.

Processing for Electron Microscopy

Follicles that were embedded in collagen gels were collected from the culture medium by pipette and directly transferred to fixative. Later transfers were done by pipette. Follicles not in a gel were collected by centrifugation. The fixative used was 2.5% glutaraldehyde (Ladd Research Industries, Inc., Burlington, VT) and 0.5% tannic acid (Mallinckrodt Inc., Science Products Div., St. Louis, MO; cat. no. 1764) (17, 18) in 0.1 M cacodylate buffer, pH 7.4. Follicles were fixed for 15 min, postfixed in 1% OsO₄ in the cacodylate buffer for 20 min, stained for 1 h in 1% aqueous uranyl acetate, dehydrated in a graded series of ethanols and then embedded directly in Epon 812 (Ladd Research Industries, Inc.). Thin sections were stained with uranyl acetate and lead citrate and examined using a Philips 201C electron microscope.

RESULTS

Thyroid follicles isolated by collagenase treatment did not have or reform a basal lamina (Fig. 1 a) under our conditions of suspension culture, a maintenance culture. We have tested whether addition to the culture medium of collagen and laminin, components of extracellular matrix, results in the formation of a basal lamina on the surface of the thyroid follicles.

Collagen

Thyroid follicles embedded in a gel of purified rat tendon (Fig. 1 b) or of purified calf skin collagen (not illustrated) did not generally develop a basal lamina although there was an occasional very short segment. Collagen fibers were observed adjacent to or in contact with the basal surface of epithelial cells.

Laminin

Segments of a basal lamina were observed on many thyroid epithelial cells when laminin was added to the medium (Fig. 1 c and d). Short segments were found within 0.5 h after the addition (Fig. 1 c) and these lengthened progressively as the time interval increased after the addition. The basal lamina was usually normal in appearance with a lamina rara and a lamina densa. Sometimes the lamina rara was not clearly visible. The width of the lamina densa was ~0.018 µm, and neither the width nor the density of the basal lamina appeared to increase with increasing time intervals after the addition of laminin.

Collagen and Laminin

The addition of collagen and laminin to the medium results in the formation of a basal lamina with a texture similar to that with laminin alone but the lamina densa appeared to be thicker, ranging from 0.023 to 0.031 µm (Fig. 1 e). The results were similar, independent of the order of the additions. Moreover, the basal lamina had a number of features similar to those in vivo, including the fact that it was continuous in crossing the boundary between cells and in spanning the gap across the mouth of a coated pit (Fig. 1 e).

In the presence of collagen and laminin, if the serum concentration was raised to 5% the basal lamina had a denser texture than in 0.5% serum, and it resembled more closely a basal lamina (Fig. 1 f) in vivo.

The basal lamina formed had a special relation to the collagen fibers. In the absence of a basal lamina the fibers tended to be in close contact with the plasma membrane. However, if laminin was added and a basal lamina was present, the collagen fibers appeared to be lifted off the plasma membrane (Fig. 1 e and f). The close association of collagen fibers with one side of the basal lamina also occurs in vivo as seen in basal lamina isolated from thyroid tissue (2).

Specificity of the Site of Deposition of the Basal Lamina

There was specificity in the site of deposition of a basal lamina. It was deposited on the basal surface of the epithelial cells, and not on the lateral surface (Fig. 1 e). It was not deposited on the apical membrane with microvilli that happened to be exposed to the medium. Finally, although a newly formed basal lamina might extend over the bases of many cells, some cells, apparently healthy, did not have a basal lamina even after several days exposure to laminin.

DISCUSSION

The formation of a basal lamina has been studied primarily during the development of various epithelia. Some epithelia like that in the submandibular gland will deposit a basal lamina in the absence of other cell types, and in the absence of extracellular matrix (4). A number of different epithelia such as that from cornea (15), mammary gland (7), and epidermis...
(12) will not form a basal lamina in the absence of at least some substance from extracellular matrix, but will in the presence of collagen. Finally, it has been reported that fibronectin may allow the formation of a basal lamina in the developing mouse tooth organ (6).

We studied the requirements for the deposition of a basal lamina in a differentiated cell, thyroid epithelium. The addition of purified collagen clearly does not suffice for the formation of a basal lamina, but a basal lamina will form in the presence of a small concentration of laminin.

**Source of Basal Lamina Constituents In Vivo**

The source of the laminin in vivo has not been studied in the thyroid gland. However, one possible source seems to be endothelial cells (9). Endothelial cells are present in abundance for a longer time interval than in 0.5% serum since the density appears to be due to the fact that the cultures were in 5% serum and C cells. Our data do suggest, however, that there may cultures with the exception of trace amounts of endothelial formation of a basal lamina (1).

Source of other constituents of the basal lamina is probably the epithelial cell itself since all the cells are epithelial in our cultures with the exception of trace amounts of endothelial cells and C cells. Our data do suggest, however, that there may be a contribution from serum because the density of the basal lamina formed in the presence of collagen plus laminin appears to be greater in 5% serum than in 0.5% serum. This does not appear to be due to the fact that the cultures were in 5% serum for a longer time interval than in 0.5% serum because the density and width of the basal lamina did not appear to change with time in 0.5% serum.

**Specificity of the Site of Deposition**

There is considerable specificity in the site of deposition of the basal lamina and the deposition resembles that in vivo. The site of deposition is the basal surface of some epithelial cells. The deposition is continuous across the margin of the neighboring cells and across the mouth of coated pits. It remains at the basal surface of cells and normally does not cover the lateral surfaces, nor the apical surface in the occasional cell in which that surface is exposed. Moreover, it does not cover the basal surface of all of the epithelial cells that are present in the normal position and orientation in the follicle. In this respect it is also similar to the situation in vivo in which one finds, occasionally, that the basal surfaces of epithelial cells of neighboring follicles are close together without an intervening basal lamina (13). Our results suggest that even in vivo there may be two classes of thyroid epithelial cells, some of which can form a basal lamina and some of which cannot.

**Rapid Formation of the Basal Lamina**

One interesting feature of the basal lamina is that it forms rapidly. We see short segments within a half hour. In this respect our system resembles the formation of a basal lamina in the embryonic submandibular gland in which a basal lamina forms within 2 h (4). The formation of basal lamina first in the form of short segments has also been observed in epidermis (12), and the results are also similar in that the length of the segments increases as the period of incubation gets longer. The observations on epidermis differ in that the time interval before appearance of the basal lamina is approximately 1 wk, much longer than in thyroid follicles.

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