EPIDERMAL CELLS ADHERE PREFERENTIALLY TO TYPE IV (BASEMENT MEMBRANE) COLLAGEN

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ABSTRACT

Epidermal cells from adult guinea pig skin attach and differentiate preferentially on substrates of type IV (basement membrane) collagen, compared to those of types I–III collagen. In contrast, guinea pig dermal fibroblasts attach equally well to all four collagen substrates. Fibronectin mediates the attachment of fibroblasts but not of epidermal cells to collagen.

KEY WORDS epidermal cells · adhesion · collagen · basement membrane · differentiation

Several chemically and genetically distinct collagens are found in mammalian species. Types I and III collagen are widely distributed (14). Type II collagen is found only in cartilage (13) and type IV in basement membranes (9). The matrices that these collagens form differ in appearance and physical characteristics. Collagen substrates enhance the attachment (10), growth (2), and differentiation (5) of various cell types. Such observations suggest that the interaction of cells with collagen may have important functions in vivo. Some clue to the mechanism(s) operative in this interaction came from recent studies (10, 11) which indicate that fibroblasts do not bind directly to collagen substrates but that a membrane protein (the collagen-cell attachment protein) mediates the attachment of cells to collagen substrates. This protein (or proteins) is similar or identical to CSP (23), LETS protein (7) and cold insoluble globulin (15, 19), and has been named fibronectin (18). Fetal calf serum used in cell culture is a rich source of fibronectin (10).

It seemed conceivable to us that collagen-cell interactions depend not only on mediator proteins but also on either the chemical characteristics of the collagen substrate or on the nature of the attaching cell. To test this hypothesis, we compared the attachment of two functionally diverse cell types, fibroblasts and epidermal cells, to various collagen substrates and their dependence on fibronectin for the mediation of attachment. In contrast to fibroblasts, epidermal cells from postembryonic animals are difficult to culture in vitro unless conditioned media and collagen substrates (8) or feeder layers of fibroblasts (17) are used. We show here that epidermal cells differ from fibroblasts in their attachment to collagen substrates and that epidermal cells attach best to type IV collagen whereas fibroblasts attach equally well to all collagen types. Further, we demonstrate that fibronectin enhances the attachment of fibroblasts to collagen but does not affect epidermal cell attachment.

MATERIALS AND METHODS

Collagen Substrates

Type I collagen was prepared from the skins of lathyritic rats (1), type II from a rat chondrosarcoma (20), type III from fetal calf skin (4), and type IV from a murine sarcoma (16, 22). The purity and identity to standards of these collagens was confirmed by sodium dodecyl sulfate (SDS) gel electrophoresis (12) and amino acid analysis. The various collagens (1 mg/ml in 0.5 M acetic acid) were stored at −20°C. To study the attachment of cells, the stock solutions of collagen were diluted to 10 μg/ml with water, and 1 ml of this solution was

added to a 35 × 10 mm bacteriological dish (Falcon, Becton, Dickinson & Co., Cockeysville, Md.) and allowed to dry in air at room temperature. For long-term culture of cells, the dishes were coated with 100 μg of collagen and sterilized by exposure to ultraviolet light for 12 h.

Cells

Adult guinea pig dermal fibroblasts (provided by L. Wahl, National Institute of Dental Research) were obtained from outgrowths of dermal explants grown in Dulbecco Vogt's medium supplemented with 10% fetal calf serum and were cultured in the same medium in T-75 flasks. For use in attachment assays, fibroblasts were removed from the flasks with a solution of 0.1% trypsin and 0.1% EDTA in 0.15 M NaCl, 0.02 M sodium phosphate, pH 7.4 (phosphate-buffered saline), washed and resuspended in Eagle's Minimal Essential Medium (MEM).

Epidermal cell suspensions were obtained by the method of Stingl et al. (21). Keratome sections of guinea pig skin were floated on 0.5% trypsin in phosphate-buffered saline for 90 min at 37°C. Dermis and epidermis were separated with fine forceps, and the epidermis was agitated with a glass rod in TC 199 medium containing 20% fetal calf serum to disperse the cells. Cells were centrifuged, washed twice, and suspended in MEM without serum. Viabilities were in excess of 90% as measured by trypan blue exclusion.

Attachment Assays

Collagen-coated bacteriological dishes were preincubated for 1 h at 37°C with 1 ml of Eagle's MEM containing 200 μg/ml serum albumin, in the presence (up to 10%) or absence of normal human serum or fetal calf serum, or purified fibronectin. Cells (10^5 fibroblasts or 4–6 × 10^5 epidermal cells) were added in 50 μl of medium and incubated for 90 min in the case of fibroblasts and 180 min in the case of epidermal cells, unless otherwise stated. Nonattached cells were removed by gently rinsing the dishes with phosphate-buffered saline. Although some epidermal cells attach by 180 min, these cells take longer to spread on the substrate than fibroblasts, necessitating gentle rinsing of the dishes. Attached cells were removed with 0.1% trypsin and 0.1% EDTA in phosphate-buffered saline and counted electronically with a Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.).

Fibronectin

Fibronectin was prepared from normal human serum by absorption to a column of denatured type I collagen covalently bound to Sepharose 4B (6, 3). Serum was left in contact with the absorbent overnight at 4°C. The column was washed with several volumes of MEM. Fibronectin was eluted from the absorbent with 1 M KBr, 0.05 M Tris-HCl, pH 5.3, dialyzed against MEM to remove KBr, and stored at −20°C. Fibronectin prepared in this fashion consisted of a major protein component with the mobility on SDS gel electrophoresis of a protein of ~400,000 daltons. After reduction with mercaptoethanol, the major band had a mobility of ~200,000 daltons, characteristic of fibronectin (23).

Electron Microscopy

Epidermal cell cultures were fixed directly on collagen-coated culture dishes (or on collagen-coated permanox cover slips) and processed as previously described (25).

RESULTS

The attachment properties of guinea pig epidermal cells differ from those of fibroblasts. Fig. 1 shows the time course for attachment of epidermal cells and fibroblasts to the different types of collagen and to bacteriological plastic in the ab-

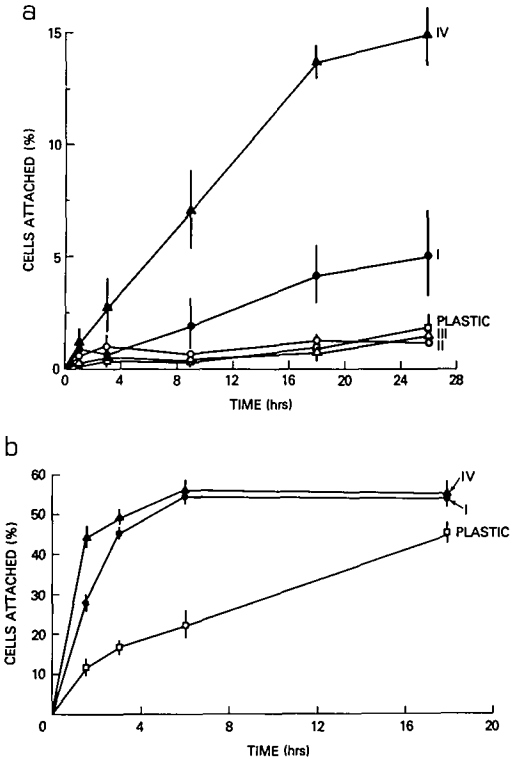

FIGURE 1 Time course of attachment of (*a*) epidermal cells and (*b*) fibroblasts to collagen-coated dishes. Cells were plated on 35-mm bacteriological dishes coated with 10 μg of collagen (type I, II, III, or IV) in MEM without serum. After various times, unattached cells were rinsed off and the attached cells were removed by trypsinization and counted electronically. Solid bars represent the SEM.

sence of serum. Replicate samples from two experiments show that epidermal cells attached slowly to the various substrates (Fig. 1 *A*). Attachment reached a maximum by 24 h of incubation, at which time attachment to type IV collagen-coated dishes was four- to five-fold higher than to type I collagen. Little attachment was seen on types II or III collagen or on bacteriological plastic alone. A maximum of 25% of the epidermal cells attached to type IV collagen-coated dishes in any of these experiments. Fibroblasts, in contrast to epidermal cells, attached rapidly to all types of collagen. Attachment to types I and IV collagen

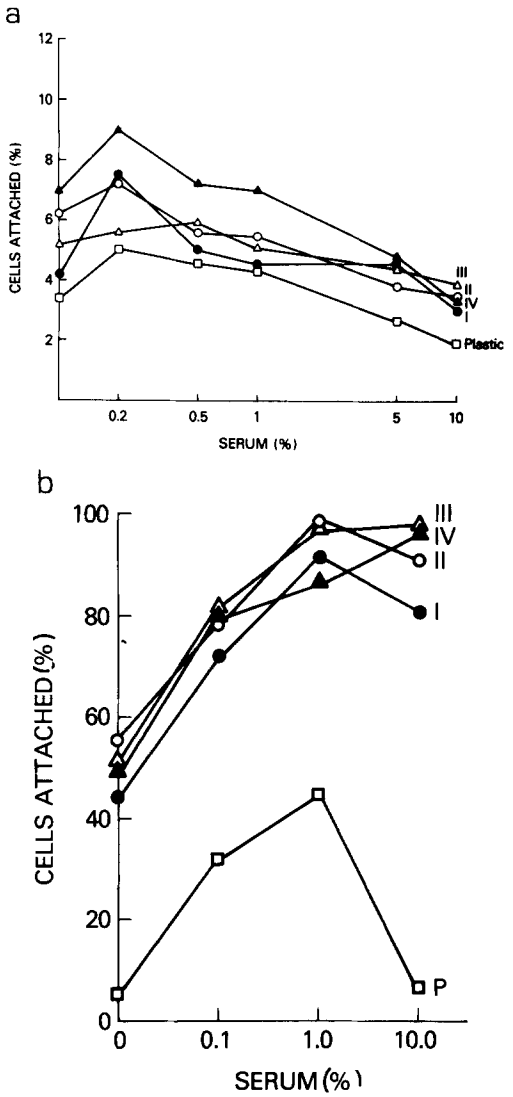

FIGURE 2 The effect of normal human serum on the attachment of (*a*) epidermal cells and (*b*) fibroblasts to collagen-coated dishes. 35-mm bacteriological dishes coated with 10 μg of type I, II, III, or IV collagen were preincubated for 1 h with varied amounts of normal human serum. Cells were added and incubated for a further 90 min in the case of fibroblasts and 180 min in the case of epidermal cells. After incubation, attached cells were removed by trypsinization and counted electronically. Each point represents the mean of duplicate assays.

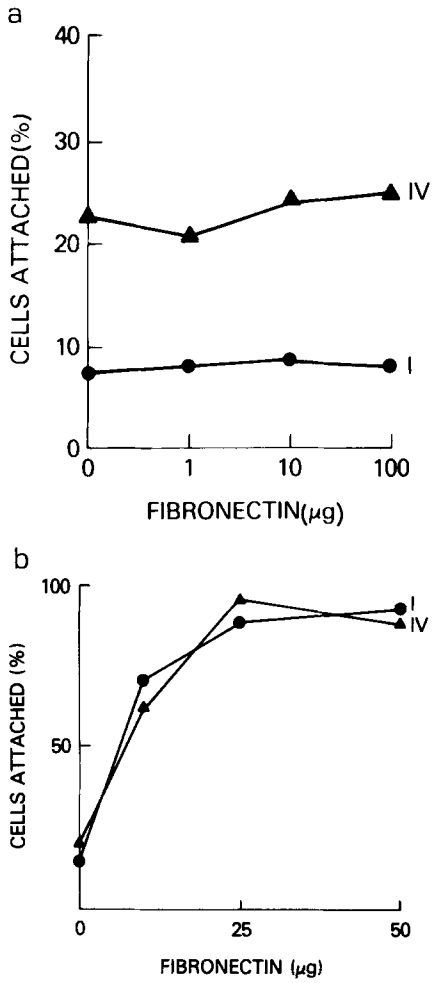

FIGURE 3 Effect of fibronectin on the attachment of (*a*) epidermal cells and (*b*) fibroblasts to collagen-coated dishes. 35-mm bacteriological dishes coated with 10 μg of types I or IV collagen were preincubated with 1 ml of MEM containing varied amounts of fibronectin purified by affinity chromatography on type I collagen-Sepharose 4B. Cells were added and incubated on the dishes for a further 90 min in the case of fibroblasts and 18 h in the case of epidermal cells. After incubation, cultured cells were removed by trypsinization and counted electronically. Each point represents the mean of duplicate assays.

was maximal by 6 h of incubation (Fig. 1 *B*). Cells attached as well to types II and III collagen as to types I and IV (data not shown). Fibroblasts attached more slowly to bacteriological plastic, although by 18 h the level of attachment to plastic was similar to that observed on collagen-coated dishes. It should be noted that, in these attachment assays, there is considerable day to day variation, although the trends remain constant.

Fig. 2*A* shows the effect of normal human

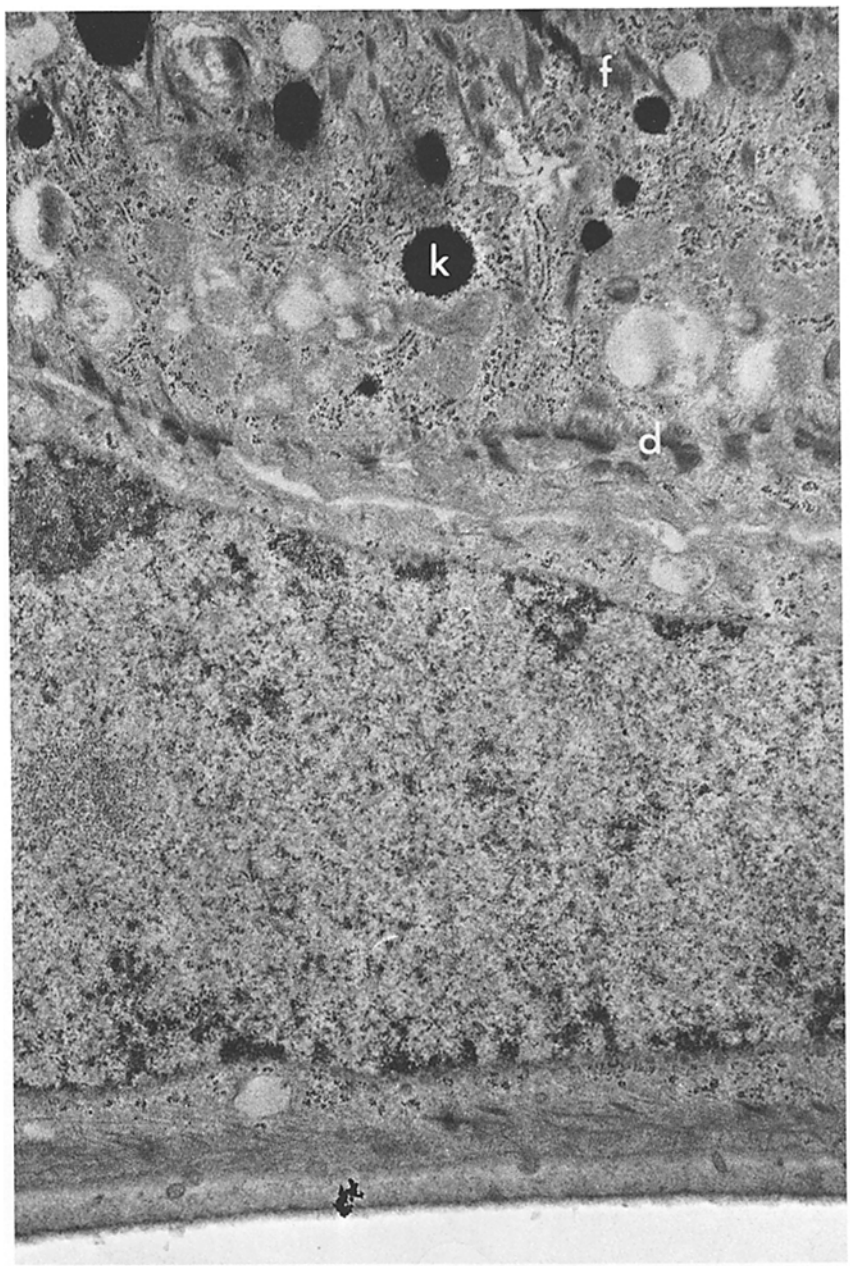

FIGURE 4 Electron micrograph of epidermal cells in a 12-d culture grown on type IV collagen-coated permanox plastic in MEM supplemented with 10% fetal calf serum. Note the presence of desmosomes (*d*), keratohyaline-like granules (*k*), and keratin-like filaments (*f*). Original magnification, × 6,800.

serum on the attachment of epidermal cells in a 3-h incubation experiment. A 3-h incubation was chosen as the minimum time required for significant levels of attachment. By 18 h, the serum-dependent curves looked similar to those at 3 h but had higher levels of attachment. Serum stimulated attachment at low concentrations but inhibited at higher concentrations. Fetal calf serum had no effect. The attachment of fibroblasts to the four types of collagen was stimulated by serum at all concentrations (Fig. 2*B*).

To assess the cells's dependence on fibronectin (a serum constituent) for attachment to collagen substrates, the collagen-coated dishes were preincubated with varied amounts of purified fibronectin. Fig. 3*B* shows that the attachment of fibroblasts to types I and IV collagen is enhanced by the addition of fibronectin, whereas the attachment of epidermal cells is unaffected by the preincubation of the collagen-coated dishes with fibronectin (Fig. 3*A*).

Fibroblasts grow to confluent monolayers equally well on all types of collagen in the presence of 10% fetal calf serum (data not shown). Epidermal cells plated on type IV collagen-coated dishes formed clusters in the presence of 10% fetal calf serum and remained viable after 3 wk of culture. In the absence of fetal calf serum, the epidermal cells rounded up and floated free after several days. Within 12 d of culture on type IV collagen, the clusters of epidermal cells formed multilayers which assumed the appearance of squamous epithelia, exhibiting desmosomes, keratin-like filaments and keratohyaline-like granules (Fig. 4).

DISCUSSION

The attachment properties of epidermal cells differ from those of fibroblasts, which adhere rapidly to all types of collagen. Epidermal cells attach slowly to collagen substrates and show a marked preference for attachment to type IV collagen. The attachment of fibroblasts to collagen is stimulated by normal human serum as well as fetal calf serum and by purified fibronectin, while attachment of epidermal cells is stimulated only by low concentrations of normal human serum but is inhibited by higher concentrations. Purified fibronectin has no effect on the attachment of epidermal cells to collagen substrates; it may be that these cells synthesize an alternate attachment protein, which is specific for type IV collagen. The slow rate of adherence of epidermal cells may be explained by the necessity of *de novo* synthesis of such a protein. In our experiments, only a small proportion of cells attached to the collagen-coated dishes (<25%), which may reflect the small proportion of cells associated with the basement membrane in vivo. Others have reported low plating efficiencies for epidermal cells in vitro (17).

In vivo, the basal cells, which represent a small proportion of the cells in the epidermis, are arranged in close association with the basement membrane, a matrix rich in type IV collagen (24). The basal cells divide and ascend in the epidermis, forming squamous epithelia. We have shown that, after attachment to type IV collagen substrates in vitro, epidermal cells form multilayered squamous epithelia and differentiate in the presence of serum. The localization of certain cells to their specific matrices may be determined by their ability to interact specifically with the type of collagen present in the matrix. Such specific collagen-cell interactions may direct the growth and differentiation of cells and the form and function of tissues.

We wish to acknowledge the excellent technical assistance of Mrs. M. Gullino, Mr. E. W. Bere and Mr. H. Schaefer, and thank Dr. P. Steinert for useful discussions.

Received for publication 20 July 1978, and in revised form 3 October 1978.

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