The Hyaluronate Receptor Is Preferentially Expressed on Proliferating Epithelial Cells

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Abstract. In the present study, we have examined the distribution of the hyaluronate receptor as well as hyaluronate itself in a variety of adult tissues. The hyaluronate receptor was localized with a monoclonal antibody, termed K-3, while hyaluronate was localized using proteolytic fragments of cartilage proteoglycan. Staining with the K-3 monoclonal antibody revealed that the hyaluronate receptor was present in a variety of epithelia including the skin, cheek, tongue, esophagus, vagina, intestines, oviduct, and bladder. However, it was notably absent from epithelial cells of the cornea and stomach as well as from endothelial cells of blood vessels. When present, the hyaluronate receptor was preferentially located in regions of active cell growth, such as in the basal layers of stratified epithelium and at the base of the crypts of Lieberkuhn in intestinal epithelium. A similar phenomenon was observed in cultured 3T3 cells. Cultures of 3T3 cells that were actively proliferating were found to have greater amounts of the receptor than their nonproliferating counterparts. When the various tissues were examined for hyaluronate, it was found to have a widespread distribution, being present in most of the basement membranes and between the cells in stratified epithelium. Indeed, in many cases, the distribution of hyaluronate closely paralleled that of the hyaluronate receptor. These results suggest that the interaction between hyaluronate and its receptor is involved in cell-to-substratum adhesion.

THE hyaluronate receptor is a transmembrane glycoprotein of M_r 85,000 that binds to hyaluronate in the extracellular matrix. The interaction between the receptor and hyaluronate has been shown to be responsible for a type of cell-to-cell adhesion (21, 24, 25). The aggregation of a variety of cells can be inhibited by enzymes that degrade hyaluronate (24). Binding studies have revealed that the receptor recognizes a six-sugar sequence of hyaluronate. It will also bind to chondroitin sulfate with a low affinity, but not to other glycosaminoglycans (22, 23, 26). And finally, the hyaluronate receptor appears to be associated with actin filaments of the cytoskeleton, which may account for some of the effects that hyaluronate has on cell movement (13).

In a recent study (7), we examined the distribution of the hyaluronate receptor in lung tissue using a monoclonal antibody (K-3 mAb) to the receptor of hamsters (27). We found that the hyaluronate receptor was present on the bronchial epithelial cells. In addition, we used a probe derived from cartilage proteoglycan to localize hyaluronate itself, and found that it was prominent in the basement membrane of the bronchial epithelium. These observations prompted us to speculate that the receptor was involved in the attachment of the epithelium to the substratum and may help to organize the actin filaments within the epithelial cells (7).

The original intent of the present study was to determine if hyaluronate and its receptor were distributed in a similar fashion on other epithelia. To this end, we examined a variety of adult tissues, and found that in most cases, both of these components were closely associated with each other, which is consistent with its postulated role in cell-to-substratum adhesion.

In the course of this study, it became apparent that the hyaluronate receptor was preferentially expressed on epithelial cells undergoing active cell division. This situation was particularly prominent in the intestinal epithelium where cells lining the base of the crypts of Lieberkuhn contained the receptor, while those further away, toward the lumen and lining the villi did not. This unusual distribution suggested that the expression of the receptor was under developmental control and may be an important factor in modulating adhesion of the cells as they mature and differentiate. Furthermore, the presence of the hyaluronate receptor might serve as a marker for carcinomas that are derived from actively growing epithelia.

Materials and Methods

Preparation of Biotinylated Probes

The biotinylated K-3 mAb (b-K-3 mAb)[1] was prepared as described previously (7) and is briefly outlined as follows. The K-3 hybridoma was grown

1. *Abbreviations used in this paper*: b-mIgG, biotinylated mouse IgG; b-K-3 mAb, biotinylated K-3 mAb; b-PG, biotinylated fragments of proteoglycan; CMF-PBS, calcium- and magnesium-free PBS.

in the peritoneal cavity of BALB/c mice. The resulting ascites fluid was collected and the IgG fraction was purified by affinity chromatography on protein A coupled to Sepharose CL-4B using a commercially available buffer system (Monopure; Pierce Chemical Co., Rockford, IL). The purified antibody was then biotinylated using a modification of the method of Updyke and Nicolson (29). The concentration of the K-3 mAb was adjusted to 1.0 mg/ml in 0.1 M Hepes, pH 8.0, and to each milliliter of this solution was added 0.2 ml of 1 mg/ml sulfosuccinimidyl 6-(biotinamide)hexanoate (Pierce Chemical Co.) in 0.1 M Hepes buffer, pH 8.0. After incubating for 30 min at room temperature, the solution was extensively dialyzed against calcium- and magnesium-free PBS (CMF-PBS) followed by 0.15 M NaCl. The resulting material was stored at −70°C until use.

As a control for the b-K-3 mAb, the IgG fraction from whole mouse serum (Sigma Chemical Co., St. Louis, MO) was isolated and biotinylated in identical fashion to that described above. This preparation is referred to as b-mIgG.

The biotinylated fragments of proteoglycan (b-PG) were prepared according to the method previously described (7) and is briefly outlined as follows. Bovine nasal cartilage was extracted overnight in 4 M guanidinium HCl, 0.5 M Na acetate, pH 5.8. After centrifugation, the supernatant was dialyzed and lyophilized. This material was then dissolved in 0.1 M Hepes, 0.1 M Na acetate, pH 7.3 and briefly digested with trypsin. The digestion was terminated after 2 h by the addition of soybean trypsin inhibitor. The resulting proteolytic fragments were coupled to biotin by the addition of 0.1 mg sulfosuccinimidyl 6-(biotinamide)hexanoate for each milligram of protein using the methods of Updyke and Nicolson (29). And finally, the hyaluronate binding fraction was isolated by affinity chromatography on hyaluronate coupled to Sepharose (20).

Tissue Preparation and Sectioning

Adult Syrian hamsters (Charles River Breeding Laboratories, Inc., Wilmington, MA, and Bio Breeders Inc., Watertown, MA) were used throughout this study. The animals were anesthetized by injection of sodium pentobarbital (5 mg/100 mg body wt) and the systemic circulation was rinsed by cardiac perfusion with 25 ml of CMF-PBS at room temperature. The hamsters were then perfused through the aorta with ~200 ml of ice-cold 4% paraformaldehyde in CMF-PBS for 20 min. After perfusion, the various organs were dissected from the animals, and postfixed in 4% paraformaldehyde in CMF-PBS for 1–4 h depending on the tissue. After fixation, the tissues were rinsed several times in CMF-PBS for 12 h at 4°C to completely remove the paraformaldehyde.

The tissues were then embedded in polyester wax according to the method of Kusakabe et al. (12). For this the tissues were dehydrated through graded steps of ice-cold ethanol, and then equilibrated with a 50% (vol/vol) solution of polyester wax (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) in absolute ethanol at 38°C for 1 h. The tissues were then incubated in 90% polyester wax, 10% absolute ethanol at 37°C for 30 min to 4 h depending upon the tissue (skin required a longer infiltration time). Tissues were finally embedded in the 90% wax/10% ethanol solution using plastic embedding trays (Polysciences, Inc., Warrington, PA) that were immersed in an ice bath. The blocks were cut into 10-μm-thick ribbons on a microtome in a cold room at 4°C. The ribbons were spread on a water bath at 4–10°C containing a small amount of Triton X-100 to reduce the surface tension. The sections were picked up on glass slides that had been coated with egg albumin (16) and then allowed to dry slowly at 4°C. The sections could be stored at 4°C for several months without significant loss of immunoreactivity.

Immunohistochemical Staining with b-K-3 mAb and b-PG

The polyester wax sections were dewaxed through a graded series of ethanol solutions (5 min each step), followed by an incubation in CMF-PBS for 5 min (12). The sections were then immersed in 10% H_2O_2 solution for 5 min to inhibit endogenous peroxidase activity, followed by washing in two changes of distilled water for 5 and 3 min. The sections were incubated with the various biotinylated probes for 1 h at room temperature. The b-K-3 mAb and b-IgG were used at a final dilution of 4 μg/ml and the b-PG at a dilution of 2 μg/ml. Each probe was dissolved in a solution of 10% calf serum in CMF-PBS. After two washes with CMF-PBS, the sections were incubated for 15 min at room temperature with peroxidase-conjugated streptavidin (Kirekegaard & Perry Laboratories, Inc., Gaithersburg, MD) at a dilution of 1:500 in 10% calf serum in CMF-PBS. Sections were washed in CMF-PBS and the peroxidase activity was demonstrated by adding the insoluble substrate for peroxidase, 3-amino-9-ethyl carbazole (6). The reaction was microscopically monitored at low magnification and stopped by washing the sections in distilled water. The reaction time was ~5–10 min for b-PG and 15–60 min for b-K-3 mAb and b-IgG. In each case, the control sections (i.e., b-mIgG or b-PG preadsorbed with 100 μg/ml hyaluronate) were incubated for an identical period of time. The sections were mounted in Crystal/Mount medium (Biomeda Corp., Foster City, CA) to preserve the chromogen and then a coverslip was attached by using Permount (Fisher Scientific, Columbia, MD).

Hyaluronate Binding Assay

The 3T3 cells were grown and cultured as described previously (25). Two different sets of cultures were established, one at low density in which most of the cells were still actively dividing and the other at a higher density in which most of the cell division had ceased. Both sets of cells were harvested by first incubating the cultures in 0.02% EDTA in CMF-PBS for ~10 min, then directing a stream of CMF-PBS from a Pasteur pipette against the surface of the plate. The number of cells in each suspension was determined by multiple counts on a hemocytometer. The cell suspensions were then centrifuged at low speed (600 *g*, 5 min) and the cell pellets were dissolved in 0.1% Na deoxycholate, 0.5 M NaCl, 0.02 M Tris, pH 8.0 (DOC buffer). The extracts were then incubated with varying amounts of [^{3}H]hyaluronate (0.92×10^5 cpm/μg) in a total volume of 0.5 ml (26, 28). To determine the value of the background, 200 μg of nonlabeled hyaluronate was included in some of the samples. After incubating for at least 15 min, an equal volume of saturated $(NH_4)_2SO_4$ was added to each sample followed by 50 μl of nonfat milk to act as a carrier. The mixtures were then centrifuged (9,000 *g*, 5 min) and the centrifuge tubes containing the precipitates were washed several times with 50% saturated $(NH_4)_2SO_4$. Finally, the precipitates were dissolved in 1-ml aliquots of water and then processed for scintillation counting. The results are expressed in terms of specific binding in which the background has been subtracted and all values represent the average of duplicate determinations.

Results

Distribution of Hyaluronate and Hyaluronate Receptors in the Epidermis

In our initial studies, we examined the skin from an adult hamster. When thick skin from one of the foot pads was processed immunohistochemically with the b-K-3 mAb, specific staining was apparent in the epidermis (Fig. 1 *a*). This staining was most prominent on the surfaces of keratinocytes in the basal layer and in the lower parts of the spinous layer where most of the cell division occurs. Within the spinous layer, the staining decreased in intensity as the distance from the basal layer increased. Staining was absent from the granular, transitional (lucid), and horny layers, where no cell division of keratinocytes occurs. In thin skin (Fig. 1 *e*), the receptor showed a similar but more condensed staining pattern, and was also present on the keratinocytes lining the hair follicles. Little or no staining was apparent in control sections which had been incubated with similar amounts of b-mIgG and then processed in an identical manner (Fig. 1 *b*).

The hamster skin was also stained for hyaluronate, using the b-PG probe, which specifically binds hyaluronate (7). This probe showed that hyaluronate was widely distributed throughout the integument (Fig. 1, *c* and *f*). As anticipated, hyaluronate was present throughout the dermis of both thick and thin skin and was particularly prominent in the basement membrane region adjacent to the epidermis. In addition hyaluronate was also apparent within the epidermis itself, being present between the keratinocytes of the basal and spinous layers (Fig. 1 *c*). It was greatly reduced (but not entirely absent) from the granular, transitional, and horny

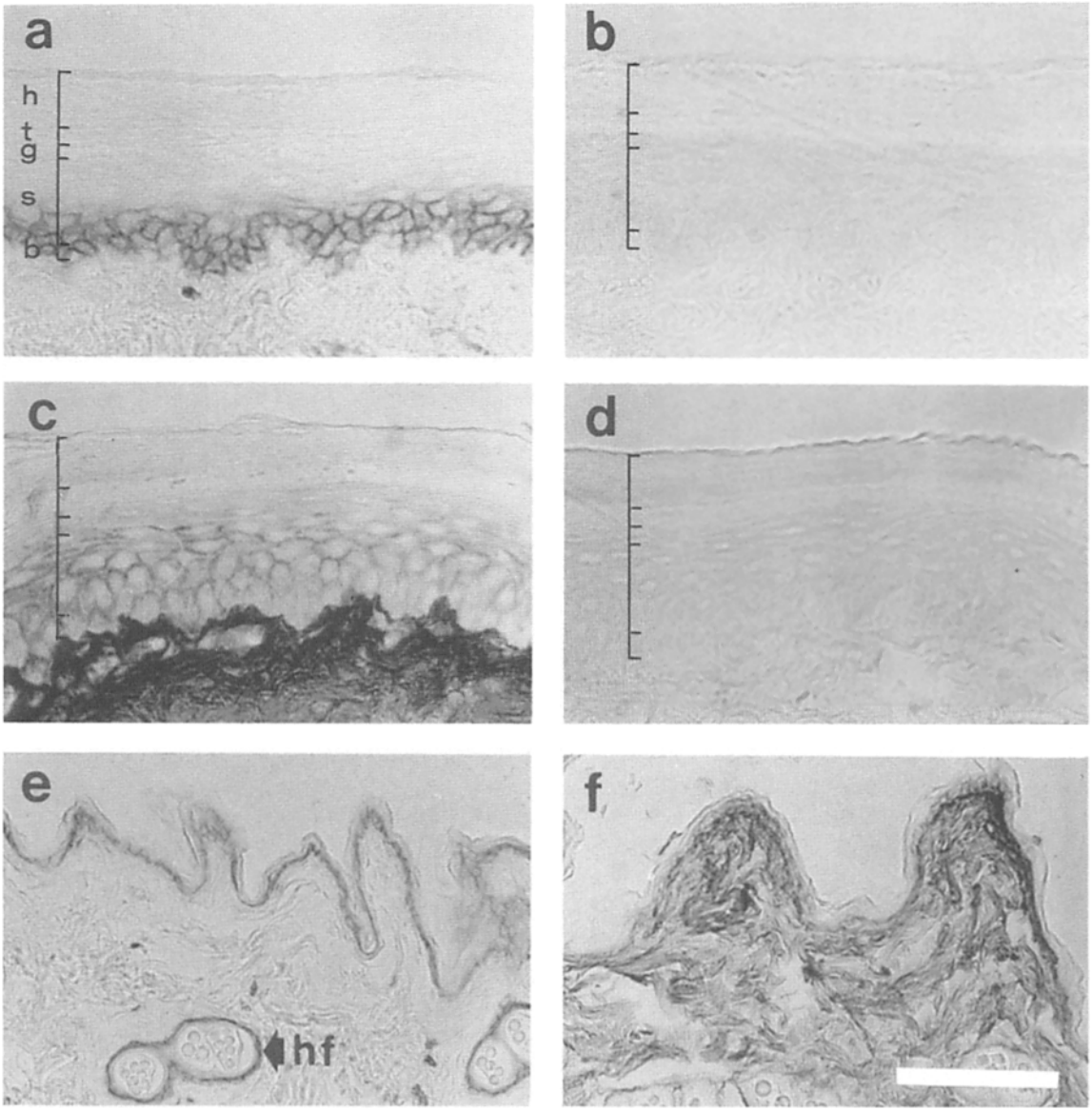

Figure 1. Histochemical localization of the hyaluronate receptor and hyaluronate in thick (*a–d*) and thin skin (*e* and *f*). (*a*) Thick skin stained with b-K-3 shows that the hyaluronate receptor is present on keratinocytes in the basal layer and the lower part of the spinous layer. The positions of the basal (*b*), spinous (*s*), granular (*g*) transitional, or lucid (*t*) and horny (*h*) layers are indicated. (*b*) Thick skin incubated with b-mIgG serves as a control and shows a lack of background staining. (*c*) Thick skin stained with b-PG reveals that hyaluronate is present between the keratinocytes of the basal and spinous layers and disappears at the level of the granular layer. Hyaluronate is also abundantly present in the dermis. (*d*) Thick skin treated with b-PG preadsorbed with hyaluronate shows a lack of specific staining. (*e*) Thin skin stained with the b-K-3 mAb shows that the hyaluronate receptor is present in the lower regions of the epidermis and extends into the hair follicles (*hf*). (*f*) Thin skin stained with the b-PG shows that hyaluronate is present in the dermis as well as between the keratinocytes in the lower regions of the epidermis. Bar, 20 μm.

layers. Thin skin showed a similar distribution of hyaluronate (Fig. 1 *f*). In each case, the staining pattern of hyaluronate and the receptor closely paralleled each other.

The staining with the b-PG probe was specific for hyaluronate since it was completely blocked if the probe was preadsorbed with hyaluronate (Fig. 1 *d*). Similarly, treatment of the sections with *Streptomyces* hyaluronidase also completely blocked the staining (data not shown), indicating that the probe specifically recognized hyaluronate.

It should be noted that the distribution of hyaluronate in the epidermis is identical to that recently reported by Tammi et al. (18).

Stratified Squamous Epithelia

The distribution of the receptor and hyaluronate in the skin was very similar to that observed in a number of other stratified squamous epithelia. Fig. 2 shows the localization of these components in the cheek (*a* and *b*), tongue (*c* and *d*), esophagus (*e* and *f*), and vagina (*g* and *h*). In every case,

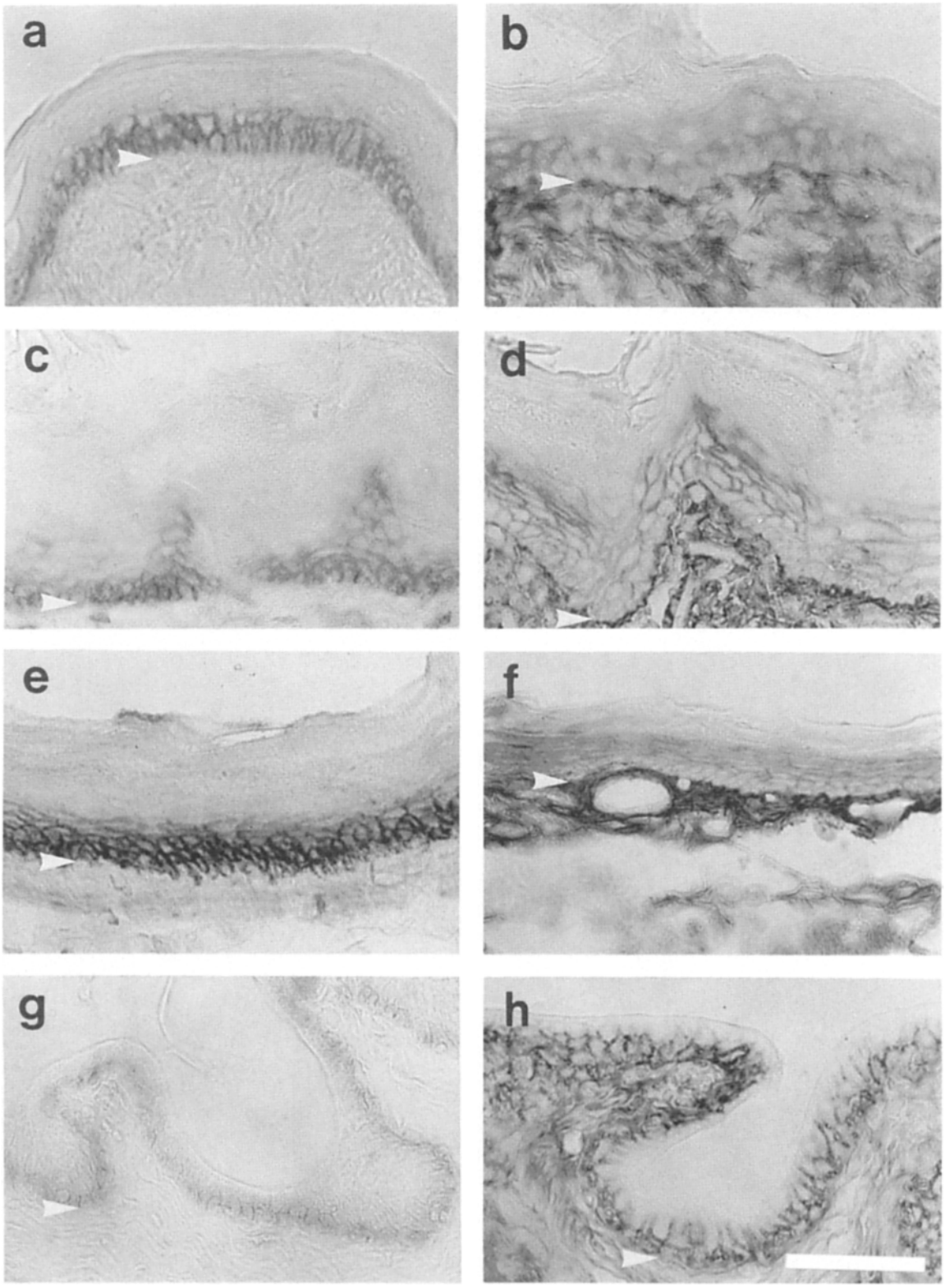

Figure 2. The distribution of the hyaluronate receptor and hyaluronate in a variety of stratified squamous epithelium. In each case the boundary between the epithelium and the basement membrane is indicated by the white arrowheads. The following tissues were stained for the receptor and for hyaluronate, respectively: (*a* and *b*) cheek; (*c* and *d*) tongue; (*e* and *f*) esophagus; and (*g* and *h*) vagina. In each case, both the receptor and hyaluronate are present in the basal layers of the epithelium. Large amounts of hyaluronate are also present in the basement membranes. Background staining (i.e., with b-mIgG or b-PG preadsorbed with hyaluronate) was either very low or absent in comparable sections from each tissue (data not shown). Bar, 20 μm.

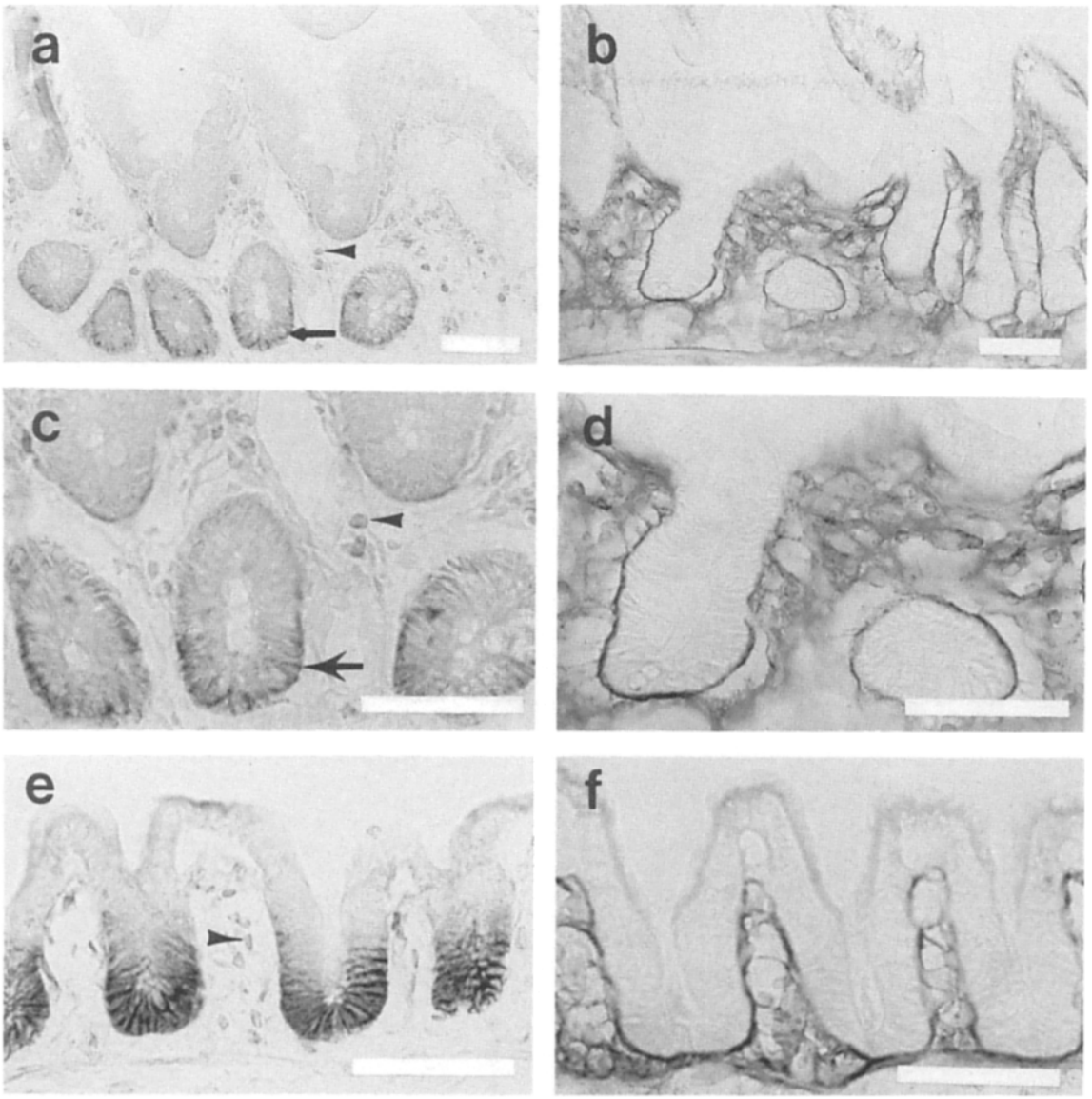

Figure 3. Histochemical localization of the hyaluronate receptor and hyaluronate in the intestinal epithelium. (*a* and *c*) Low and high magnification views of a section through the small intestines stained with the b-K-3 mAb are shown. The hyaluronate receptor is localized to epithelia lining the basal regions of the crypts of Lieberkuhn (*arrows* in *a* and *c*), but is absent from the epithelia lining the villi (*a*). In addition, some of the cells in the lamina propria also contain the hyaluronate receptor (*arrowheads* in *a* and *c*). (*b* and *d*) Low and high magnification views of the small intestines stained with the b-PG are shown. Hyaluronate is dispersed through out the lamina propria and is particularly prominent in the region of the basement membrane. However, no staining for hyaluronate was apparent between adjacent epithelial cells. (*e*) A section through the colon stained with the b-K-3 mAb shows that the hyaluronate receptor is present on epithelia at the base of the crypts of Lieberkuhn, but not on epithelial cells closer to the surface. Many of the mesenchymal cells of the lamina propria also contain the hyaluronate receptor (*arrowhead*). (*f*) Colon stained with the b-PG shows that hyaluronate is present throughout the lamina propria and is prominent in the basement membrane region. In each tissue, background staining was either very low or absent in comparable sections (data not shown). Bars, 20 μm.

both the receptor and hyaluronate were present on the deeper layers of the epithelial cells that are still undergoing cell division, but were absent from the more superficial layers. In addition, hyaluronate was a prominent component of the basement membranes in all of the tissues described above.

It is interesting to note that the epithelial cells lining the anterior cornea lacked hyaluronate receptors in the central regions (data not shown). Similarly, hyaluronate was not detectable in either the stroma or the basement membranes of the cornea. The significance of the absence of these components is unclear.

Simple Epithelia of the Gastrointestinal Tract

We extended our histochemical studies to the simple epithelia of the gastrointestinal tract. Fig. 3, *a* and *c* show that in the case of the small intestines, the receptor was present on basolateral surfaces of cells lining the base of the crypts of

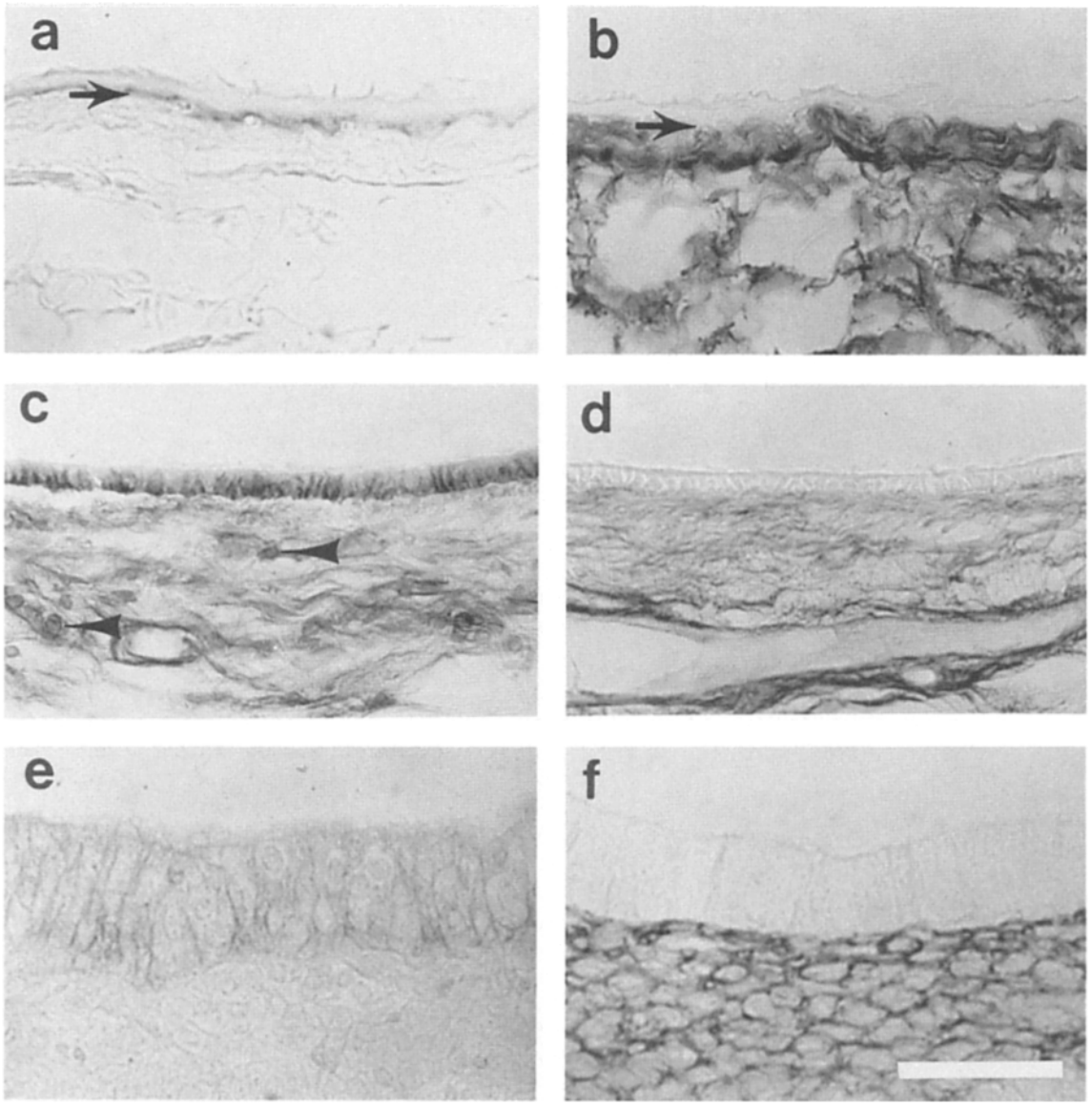

Figure 4. The distribution of hyaluronate receptors and hyaluronate in transitional, pseudostratified, and simple epithelia. (*a*) The transitional epithelium of the bladder was stained with the b-K-3 mAb and shows that the hyaluronate receptor is restricted to the basal layer of the epithelium. The arrow indicates the junction between the epithelium and the basement membrane. (*b*) Bladder epithelium stained with b-PG shows that hyaluronate is present in the basement membrane and throughout the lamina propria, but is absent from the epithelium itself. The arrow indicates the junction between the epithelium and the basement membrane. (*c*) Tracheal epithelium was stained with the b-K-3 mAb and shows that the receptor is prominent on the basolateral surfaces of the epithelial cells. In addition a large number of cells in the lamina propria also contain the receptor (*arrowheads*) and gives a diffuse staining character to this region. (*d*) The trachea stained with b-PG shows that hyaluronate is distributed throughout the lamina propria. (*e*) The oviduct stained with the b-K-3 mAb indicates that the hyaluronate receptor is present (albeit minimally) on the epithelial cells. (*f*) Oviduct processed with b-PG shows that hyaluronate is present in the lamina propria but absent from the epithelia itself. In each tissue, background staining was minimal or undetectable (data not shown). Bar, 20 μm.

Lieberkuhn, but was absent from the cells lining the mouth of the crypts and on the villi. This distribution of the hyaluronate receptor corresponded to those regions of the epithelium undergoing cell division (3).

This unusual distribution of receptors was even more pronounced in the case of the colon. Fig. 3 *e* shows that the hyaluronate receptor was prominent on epithelial cells in the crypts, but not on those cells on the surface. Again this corresponded to those regions where proliferation of epithelial cells occurred.

When sections of the small intestines (Fig. 3, *b* and *d*) and colon (Fig. 3 *f*) were stained with the b-PG, hyaluronate was shown to be present throughout the lamina propria and concentrated in the region of the basement membrane. In this latter location it could interact with the hyaluronate receptors on the basal surfaces of the epithelial cells, and is consistent with its postulated role in cell adhesion. However, no hyaluronate was apparent between the individual epithelial cells of the intestines.

It should be noted that a large number of cells in the lamina

propria also contained the hyaluronate receptor (see arrowheads in Fig. 3, *a* and *c*). These cells may correspond to macrophages that are known to have the receptor (7), however the exact identity of the cells has not been established.

Transitional, Pseudostratified, and Simple Epithelia

In the case of the transitional epithelium of the bladder (Fig. 4 *a*), the hyaluronate receptor was apparent only on the most basal layer of cells and was absent from the remaining superficial layers. In a similar fashion, hyaluronate was present in the basement membrane region and below, but did not extend into the epithelium itself (Fig. 4 *b*).

Hyaluronate receptors were also detected on the pseudostratified epithelium of the trachea (Fig. 4 *c*) and the simple columnar epithelium of the oviduct (Fig. 4 *e*). In the case of the trachea, there were a large number of cells in the lamina propria that were also positive for the receptor. In the oviduct, the staining was very weak, being considerably less than that observed with either the stratified epithelium or the simple columnar epithelium associated with the intestines. In both cases, hyaluronate was distributed uniformly throughout the lamina propria.

Other types of simple epithelia did not contain detectable amounts of the receptor. These include the epithelial cells lining the stomach, mesothelial cells lining the pleural, cardiac, and peritoneal cavities, and endothelial cells lining blood vessels (data not shown).

Quantitation of Hyaluronate Receptors on Dividing vs. Nondividing 3T3 Cells

The histochemical studies described above have suggested that the presence of the hyaluronate receptor is closely associated with actively dividing epithelial cells. We therefore decided to determine if a similar phenomenon could be detected in vitro with cultured cells. Swiss 3T3 cells were selected for this experiment since these cells display the property of contact inhibition of growth such that at high density, cell division decreases. In addition, recent studies (8) have shown that the K-3 mAb totally inhibits the [^{3}H]hyaluronate binding activity of detergent extracts of 3T3 cells, indicating that all of the binding is due to the M_r-85,000 hyaluronate receptor rather than to some other hyaluronate-binding molecule. For this experiment, one set of 3T3 cell cultures were harvested at low density during the logarithmic growth phase, while another set was harvested at a high cell density in which most of the cell division had ceased. Both samples were then assayed in parallel for [^{3}H]hyaluronate binding activity. As shown in Fig. 5, on a per cell basis, the growing 3T3 cells had from two to three times the amount of binding activity as the nongrowing cells. Scatchard analysis of this data showed that the K_ds were essentially identical, suggesting that endogenous levels of hyaluronate could not account for this difference. While these results are not as dramatic as those obtained by histochemical staining, they are consistent with the possibility that the expression of the hyaluronate receptor depends upon the growth state of the cells.

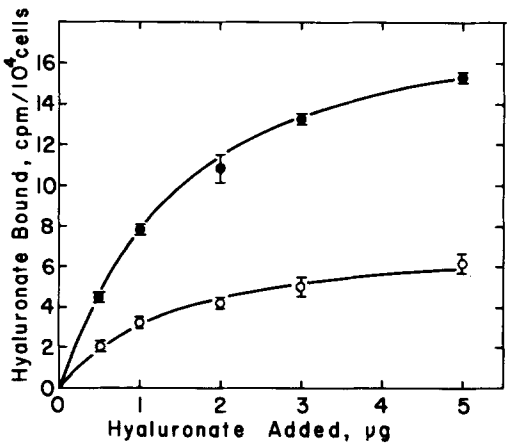

Figure 5. Binding of isotopically labeled hyaluronate to subconfluent (growing) and confluent (nongrowing) cultures of 3T3 cells. Both the growing (●) and nongrowing (○) 3T3 cells were extracted with DOC buffer, and aliquots of this extract were mixed with the indicated amounts of [^{3}H]hyaluronate. The specific binding was determined as described in Materials and Methods. The growing 3T3 cells had two to three times the hyaluronate binding activity of the nongrowing cells.

Discussion

Several lines of evidence suggest that the hyaluronate receptor in epithelial tissue is preferentially expressed on proliferating cells as compared with their nonproliferating counterparts. First, immunohistochemical staining of the small intestines and colon showed that the receptor was readily apparent on dividing epithelial cells in the base of the crypts of Lieberkuhn, but absent from the nondividing epithelial cells near the mouth of the crypts and on the villi. Secondly, in a variety of stratified squamous epithelia (epidermis, tongue, cheek, esophagus, etc.), the hyaluronate receptor was present on cells in the basal layers where most of the cell division occurs, but absent from the more superficial layers containing nondividing cells. And finally, when the amount of the receptor associated with cultured 3T3 cells was examined using the [^{3}H]hyaluronate binding assay, subconfluent cultures of rapidly dividing cells had a greater amount of the receptor as compared with confluent cultures of quiescent cells. Taken together, these results indicate that the expression of the hyaluronate receptor is associated with cell proliferation.

However, the hyaluronate receptor was absent from several types of epithelia, such as mesothelial cells and the epithelial cells lining the cornea as well as the stomach. Since cell division clearly occurs in these locations, the lack of receptors indicates that the relationship between hyaluronate receptors and cell division is not a universal feature of all epithelia. Similarly, no receptors could be detected on endothelial cells of blood vessels even though a number of studies have shown that these cells have hyaluronate binding activity (5, 14). The reason for this discrepancy is unclear.

The hyaluronate receptor may serve several functions in maintaining the integrity of the epithelium. One such function is mediating the attachment of epithelia to the basement membrane. This possibility is consistent with the fact that hyaluronate was present in the basement membranes of most epithelia, where it could readily interact with the hyaluronate receptors on the basal surfaces of epithelial cells. Another possible function of the hyaluronate receptor is to help maintain the cortical mat of actin filaments which is present

immediately adjacent to the plasma membrane of many epithelial cells (10). Previous studies have shown that the hyaluronate receptor interacts with actin filaments (13). Thus, it could provide a transmembrane bridge between the hyaluronate in the basement membrane and actin filaments in the cytoskeleton. Such an interaction might be important for stabilizing the actin filaments in the basal regions of the cells. The integrity of this cortical mat of actin may in turn regulate protein synthesis (10). Thus, the interaction of hyaluronate with its receptor on the epithelial cells may indirectly maintain the integrity of the epithelium.

The hyaluronate receptor is a member of a group of membrane proteins that maintain the interaction between the cell surface and components of the extracellular matrix. Other members of this group includes the fibronectin receptor (integrin) (1), the collagen receptor (1), the laminin receptor (9), and the heparan sulfate proteoglycan (11). It is likely that the combined effects of each of these components determines the adhesive property of each cell type. Thus, different epithelia may have different adhesive properties depending upon the particular combination of adhesive molecules that are expressed. In this way, cells may be able to alter their adhesive properties by modulating the expression of one or more of these membrane proteins.

One example of where the differential expression of receptors may be of importance is in the normal developmental sequence of the intestinal epithelium. In a series of classical studies (2, 3), Leblond and his associates showed that essentially all division of epithelial cells in large and small intestines occurs in the basal regions of the crypts of Lieberkuhn. It is also in this location that the stem cells undergo differentiation to give rise to the various cell types present in the epithelium (adsorptive cells, Paneth cells, mucus goblet cells, etc.). After undergoing differentiation, the majority of these cells migrate out of the crypts and eventually move up to the tips of the villi where they are sloughed off. This highly dynamic process undoubtedly requires carefully controlled modulation of cell adhesion.

As we have shown in the present study, hyaluronate receptors are present on epithelial cells located at the base of the crypts. In addition, Hayashi and co-workers (11) have shown that the heparan sulfate proteoglycan present on these epithelial cells is expressed on the upper third of the crypts and on the villi. Thus, as intestinal epithelial cells mature and migrate, the hyaluronate receptor is lost and the heparan sulfate proteoglycan appears. This may reflect a developmental sequence in this epithelium such that as the cells mature there is progressive change in their adhesive properties until all adhesion to the substratum is lost at the tips of the villi where the cells can be sloughed off.

Another possible function of the hyaluronate receptor is to maintain hyaluronate filled spaces between the individual cells of thick epithelia. Recently, Tammi and co-workers have shown that hyaluronate is present between the epithelial cells in the lower layers of the epidermis (18, 19). In the present study, we have confirmed this observation and extended it to include a variety of other stratified epithelia. As suggested by Hayashi et al. (19), the hyaluronate located within the epithelium may be important in maintaining intercellular spaces, which serve as conduits through which nutrients pass from the underlying basement membrane to the cells in the more superficial layers of the epithelium. The remarkable similarity between the distribution of hyaluronate and the hyaluronate receptor suggests that the interaction between the two is involved in stabilizing the extracellular hyaluronate in this location. It also suggests that both of these components are under a similar type of control. Indeed, several studies have shown that the synthesis of hyaluronate is also much greater in proliferating cells as compared with nonproliferating cells (4, 15), again paralleling what we have observed with the hyaluronate receptor.

A final aspect of the hyaluronate receptor that deserved attention is its possible usefulness as a marker for tumor cells. Since many carcinomas are derived from epithelia that express the hyaluronate receptor, it is quite possible that the receptor is expressed on growing tumors as well. Indeed, Nemec et al. (17) have recently reported that an invasive human bladder carcinoma has hyaluronate binding activity, indicative of the receptor. In the present study, we have shown that in normal bladder epithelium of hamster, only the actively growing cells in the most basal layers express the receptor. Thus, the receptor may serve as a marker for normal or abnormal proliferating cells. Along these lines, it would be interesting to determine if other types of carcinomas also express this receptor and if so whether the relative amount of this receptor is related to invasiveness, rate of proliferation, or degree of differentiation.

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