INTRODUCTION

Epican is a heparan/chondroitin sulfate proteoglycan found on the surface of keratinocytes throughout the epidermis (Haggerty et al., 1992); its core protein is a splice variant form of CD44 (Kugelman et al., 1992). Members of the CD44 family are expressed on the surface of many epithelial, mesenchymal and hematopoietic cells (Flanagan et al., 1989; Picker et al., 1989), but expression of epican is limited primarily to stratified squamous epithelial cells and a few other epithelial cells (J. Zhou and L. M. Milstone, unpublished data). Its function is not known.

Members of the CD44 family of transmembrane glycoproteins arise from alternative splicing of a single gene (Screaton et al., 1992) and variable glycosylation. Nine exons can be spliced into a single site in the region coding the extracellular domain of the protein (Fig. 1), and various combinations of these exons have been identified by PCR analysis of mRNA (Gunthert et al., 1991; Brown et al., 1991; Cooper et al., 1992; Matsumura and Tarin, 1992; Rudy et al., 1993). To date, proteins have been identified for mRNAs corresponding to CD44H, which contains no alternative exons (Jalkanen et al., 1987; Flanagan et al., 1989; St John et al., 1990; Stamenkovic et al., 1990); CD44E, which contains exons 12-14 (Stamenkovic et al., 1991; Dougherty et al., 1991); CD44M, which contains exons 8-11 (Gunthert et al., 1991; Rudy et al., 1993); and CD44Epican, which contains exons 7-14 (Haggerty et al., 1992). Extensive N- and O-linked glycosylations are found on all forms of CD44 (Jalkanen et al., 1988; Stamenkovic et al., 1991; Haggerty et al., 1992). Chondroitin sulfate has been demonstrated on CD44H (Jalkanen et al., 1988; Stamenkovic et al., 1991; Faassen et al., 1992), CD44E (Stamenkovic et al., 1991) and epican (Haggerty et al., 1992), while heparan sulfate has been identified only on epican (Haggerty et al., 1992).

CD44 participates in cell adhesion to substratum (Carter and Wayner, 1988; Aruffo et al., 1990; Birch et al., 1991; He et al., 1994).
CD44 Epican

Fig. 1. Relationship between CD44Epican and the coding region exons of the CD44 gene. Open boxes represent exons common to all known splice variants of CD44; black boxes represent alternative exons for extracellular domain of variant forms of CD44; shaded boxes represent alternative exons for cytoplasmic domain. LP, exon coding for leader peptide. TM, exon coding for transmembrane domain. CD44H codes for the common form of CD44 expressed by hematopoietic cells and contains no alternative extracellular exons. CD44Epican codes for the heparan/chondroitin sulfate proteoglycan form of CD44 expressed by keratinocytes and includes extracellular exons 7-14 and cytoplasmic exon 19. (Adapted from Screaton et al., 1992, with permission.)

1992; Jalkanen and Jalkanen, 1992; Thomas et al., 1992) and cell-cell adhesion (Jalkanen et al., 1987; Shimizu et al., 1989; St John et al., 1990; Stamenkovic et al., 1991; Birch et al., 1991; He et al., 1992). There is a hyaluronan-binding domain near the N terminus of all forms of CD44 (Aruffo et al., 1990; Peach et al., 1993), and CD44 has been reported to bind collagen (Carter and Wayner, 1988) and fibronectin (Jalkanen and Jalkanen, 1992). In systems where it has been studied, hyaluronan is required for the cell-cell adhesive effects of CD44 (Miyake et al., 1990; Birch et al., 1991; Stamenkovic et al., 1991; Lesley et al., 1992; He et al., 1992). Yet there is controversy regarding the effect of variations in CD44 structure on hyaluronan binding: variants of the core protein that are larger than CD44H have reduced binding to hyaluronan in some systems (Stamenkovic et al., 1991) but have had no effect on hyaluronan binding in others (He et al., 1992). The ability of epican to bind hyaluronan is not known.

Hyaluronan and CD44 co-localize between cells in the epidermis (Wang et al., 1992) and, based on morphological considerations, CD44 and/or hyaluronan have been associated with epithelial cell proliferation (Alho and Underhill, 1989) or maintenance of the intercellular space (Wang et al., 1992). Before CD44 and hyaluronan were specifically localized to the intercellular space, glycoproteins and acid mucopolysaccharides in the epidermis were often considered to be components of the epidermal ‘intercellular cement’ (Mercey et al., 1968; Wolff and Schreiner, 1968). Epican is a reasonable candidate for intercellular cement since it is the most abundant form of CD44 in the epidermis (Haggerty et al., 1992) and is also a heparan/chondroitin sulfate proteoglycan. There is a large body of information implicating proteoglycans in a variety of cell-substratum and intermolecular adhesive interactions, however no heparan sulfate proteoglycan has been shown to have a role in cell-cell adhesion in the epidermis or elsewhere.

We report herein an experimental analysis of epican in cell-cell adhesion. To isolate the effect of epican from other adhesion molecules, CD44Epican was transfected into non-adhesive, non-aggregating mouse L cells, and the effect of epican expression on their ability to aggregate with each other and to adhere to keratinocytes was measured. To establish a role for epican in adhesion between normal cells, antibodies to the core protein of epican were added to a keratinocyte-keratinocyte adhesion assay. Finally, the adhesion and aggregation assays were used to determine the effect of hyaluronan and other glycosaminoglycans on epican-mediated cell-cell adhesion.

MATERIALS AND METHODS

Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Gibco (Grand Island, NY). MCDB-153 powder was from Irvine Scientific (Santa Ana, CA). $^{51}$CrNa$_2$CrO$_4$ was obtained from NEN (Boston, MA). Highly purified human hyaluronan (10$^6$ g/mole), Flavobacterium heparinum heparitinase, and Proteus vulgaris chondroitinase ABC, were obtained from Seikagaku America (Rockville, MD). The lipophilic fluorescent dye DiIC$_{18}(3)(1,1′$ dioctetyldecyl-3,3′,3′$)tetramethylindocarbocyanine perchlorate) was obtained from Molecular Probes (Eugene, OR). Testicular hyaluronidase and all other chemicals were obtained from Sigma (St Louis, MO), except where indicated. Mouse anti-human monoclonal antibodies: mAb17 specifically binds the epican form of CD44 (Haggerty et al., 1992); H-3 (provided by Eugene Butcher) binds all forms of CD44.

Cell culture

Keratinocytes, obtained from human neonatal foreskins, were grown to subconfluence in complete MCD-B-153 medium. Complete MCD-B-153 is basal medium (Boyce and Ham, 1983) supplemented as previously described (Shipley and Pittlekow, 1987). Cells of passage 2 or 3, which had been stored in liquid nitrogen were used in these studies. L-M(TK$'$) mouse fibroblasts (obtained from American Type Tissue Culture Collection and hereafter called L cells) were grown in DMEM containing 10% calf serum with antibiotics.

Adhesion assay

The cell adhesion assay was adapted from previously described assays (Bender et al., 1987; Carter et al., 1990) and is designed to measure adhesion of $^{51}$Cr-loaded cells to monolayers of keratinocytes and to minimize cadherin-mediated adhesion. Flasks of keratinocytes or L cells were labelled with Na$_2$CrO$_4$(100 Ci/ml) for 1 hour at 37°C. The cells were washed 3x with calcium/magnesium-free phosphate buffered saline (CMF-PBS), and 1x with 1% EDTA in CMF-PBS. Cells were detached with 1% EDTA in CMF-PBS, and suspended in complete MCD-B-153 containing 0.03 mM calcium and protease inhibitors: leupeptin (1 µg/ml), chymostatin (1 µg/ml), pepstatin (1 µg/ml), and phenylmethylsulfonyl fluoride (10 µM). Aliquots of cells were placed in microfuge tubes and incubated for 60 minutes at room temperature in the presence of BSA (50 µg/ml) and the added test compounds. Ninety-six-well tissue culture plates were seeded with 1x10$^5$ cells per well and grown to confluence for adhesion assays. During the preparation of the suspended cells, the 96-well plate was washed with calcium/magnesium-free PBS and switched to complete MCD-B-153 with 0.03 mM calcium for a 30 minute incubation at 37°C. Following the preliminary incubations, medium was removed from the 96-well plate and aliquots of $^{51}$Cr-loaded, suspended cells were added to the monolayers. The plate was then incubated at room temperature without agitation. After 60 minutes, nonadherent cells were removed and the wells were washed 4x with CMF-PBS by carefully applying 200 µl of wash buffer against each of the four sides of the well. Cells were lysed with 0.2 M NaOH/0.2% SDS. $^{51}$Cr in the lysate was quantitated by counting in a gamma counter (LKB, Gaithersburg, MD). Aliquots of control cells were prepared for each assay condition in order to normalize for any differences in radioactive leakage from the cells. No treatment caused more than 15% leakage during the 60 minute assay period.
**Aggregation assay**

The aggregation assay was similar to that described by St John et al. (1990). Adherent cells were detached with EDTA and washed, as described above, and either placed directly in the assay or pretreated. For hyaluronidase pretreatment, cells were incubated with testes hyaluronidase (50 μg/ml) in DMEM + 10% calf serum and protease inhibitors for 45 minutes at 37°C with occasional agitation. Cells were washed twice with CMF-PBS prior to assay. For heterotypic mixing experiments, some cells were pretreated with the fluorescent plasma membrane dye, DiIC18(3) (Honig and Hume, 1986), which was added to a final concentration of 7.5 μM during the hyaluronidase treatment. These cells were washed 3× with 300 mM sucrose in 10 mM MOPS, pH 7.2, before suspension in aggregation assay buffer. For the assay 2×10^6 cells/ml were suspended in assay buffer consisting of 150 mM NaCl, 20 mM 3-(N-morpholino)-2-hydroxypropanesulfonic acid (MOPS), 0.5 mg/ml BSA, pH 7.0. Aliquots (300 μl) of cells were mixed with an equal volume of assay buffer and rotated at 100 rpm at 4°C on a Belly dancer (Stovall Life Science, Greensboro, NC). Aggregated suspensions were fixed with 400 μl of 5% glutaraldehyde (Urushihara et al., 1976), diluted to 10 ml, and counted in a Coulter counter equipped with a 100 μm aperture (Multisizer 2, Coulter Electronic, Hialeah, FL). The electronic window was gated to recognize particles corresponding to single cells. The number of single cells counted in a fixed volume decreased as aggregation increased. Control experiments established that all cell types had maximally aggregated within 60 minutes. The designation Nt/N0 is the ratio of single cells at t=0 compared to single cells remaining at time = t (usually 60 minutes). This ratio is used in figures combining data from several independent experiments. Single cell number is used for data generated in one experiment.

**Transfection of L cells**

A 2265 bp sequence containing the entire CD44Epican coding sequence was excised from pKG1 with EcoRI (Kugelman et al., 1992). This sequence was inserted into pcDNA 1, a eukaryotic expression vector containing a CMV promoter/enhancer and SV40 and polyoma origins of replication (Invitrogen, San Diego, CA). This plasmid and pSV2neo were co-transfected into mouse L cells by a standard calcium phosphate method. The bacterial neomycin resistance gene on pSV2neo allowed the selection of stable transfectants by growth in G-418 (200 μg/ml). After two weeks, fluorescence activated cell scan (FACS) analysis indicated that about 70% of the G-418 resistant cells were expressing epican. Individual clones were isolated by limiting dilution and chosen for further analyses based on their level of epican expression.

**Fluorescence activated cell scanning (FACS) analysis**

Epican expression was monitored by FACS analysis, as described previously (Pardi et al., 1989) on a FACSsort (Becton Dickinson, San Jose, CA). Cells were harvested in the same manner as for adhesion/aggregation assays. The primary antibody was mAb17. The secondary antibody was FITC-labelled goat anti-mouse IgG (Boehringer, Indianapolis). In some experiments, 2 μg/ml of propidium iodide was added to the analyses to distinguish a small population of dead cells.

**Identification of glycosaminoglycan (GAG) chains and core proteins**

Proteoglycans were partially purified from 4 M guanidine HCl extracts of cells by sequential chromatography. The void volume from a Sephadex G-50 column was passed over a DEAE column and the high salt fraction recovered as described previously (Haggerty et al., 1992). Covalently linked glycosaminoglycans were identified by sensitivity to degradation with heparitinase (Kato et al., 1985) or chondroitin ABC lyase (Oike et al., 1980). The core protein of epican was identified on western blots performed as described previously (Haggerty et al., 1992).

---

**RESULTS**

Epican is expressed as a proteoglycan on the surface of L cells transfected with CD44Epican

Mouse L cells were stably transfected with CD44Epican cDNA, cloned and then selected by FACS analysis for surface expression of epican using the epican-specific antibody, mAb17. Some clones (Fig. 2) expressed high levels of epican (e.g. LEp-2H5) while others expressed low levels of epican (e.g. LEp-2G5 and LEp-1D7). The nature of the material expressed on the surface of transfected cells was analyzed by PAGE/immunoblotting. Total cell extracts were probed with H-3 (Fig. 3A), a monoclonal antibody that detects all forms of human CD44 and reacts more intensely on immunoblots than mAb17. Extracts from keratinocytes contained several broad bands of reactivity including one corresponding to epican at >205 kDa and another corresponding to CD44H (see Haggerty et al., 1992). Extracts from LEp-2H5 contained only the >205 kDa broad band of reactivity along with several sharp bands at approximately 205 kDa, while extracts from L cells did not react. The proteoglycan fractions of keratinocytes and LEp-2H5 cells were separated by DEAE chromatography and also probed with H-3 antibody (Fig. 3B). Only the broad band corresponding to epican remained in the extracts. Predigestion of the LEp-2H5 extract with heparitinase and, to a lesser extent, with chondroitin ABC lyase caused the appearance of a sharpened band of increased mobility at approximately 200 kDa (Fig. 3B). Immunoblots of LEp-2H5 extracts using mAb17 showed essentially identical patterns of reactivity (data not shown). Sequential digestion with both enzymes did not increase the mobility of the epican band more than digestion alone.

---

**Fig. 2. Fluorescence scanning for surface expression of epican.**

Mouse L cell fibroblasts were stably transfected with CD44Epican cDNA (LEp). Individual clones were analyzed for epican expression using the anti-epican antibody, mAb17, and a fluorescein-conjugated secondary antibody. Each panel represents a different LEp clone.
with either enzyme alone (data not shown). These findings indicate that the CD44Epican expressed by LEp-2H5 cells is a mixture of heparan sulfate or chondroitin sulfate proteoglycans and non-glycanated core protein.

**L cells transfected with CD44Epican acquire a new aggregating phenotype**

L cells do not normally aggregate, but L cells transfected with CD44Epican acquired the ability to form aggregates of 10-20 cells (Fig. 4). Cells in the aggregates were only loosely adherent and required brief fixation prior to counting. Only cells expressing epican could participate in aggregate formation. Fluorescently tagged parent L cells were mixed with epican-expressing LEp-2H5 cells. Aggregates contained only the non-fluorescent, LEp-2H5 cells (Fig. 4C and D). The fluorescent tag did not interfere with aggregation, since tagged LEp-2H5 cells were fully capable of forming aggregates (data not shown).

**Hyaluronan is required for epican-mediated cell-cell aggregation**

In early experiments with LEp-2H5 cells, we noticed that hyaluronan had a variable effect on the size of the aggregates and on the number of single cells remaining unincorporated into aggregates. We wondered whether varying amounts of endogenous hyaluronan could account for quantitative differences in spontaneous aggregation and in the response of LEp-2H5 cells to added hyaluronan. Indeed, pretreatment of LEp-2H5 cells with testicular hyaluronidase in the presence of protease inhibitors abolished their ability to aggregate (Fig. 5A); readdition of high molecular mass hyaluronan to such hyaluronidase-treated cells fully restored their ability to aggregate (Fig. 5A). The dose-dependent stimulation of aggregation by hyaluronan was detected with as little as 10 pM hyaluronan (Fig. 5B). Hyaluronan and hyaluronidase had no effect on parent L cells. Together, these data indicate a necessary role for hyaluronan in epican-mediated cell aggregation.

Other polysaccharides had no effect on aggregation of LEp-2H5 cells (Fig. 6). When added to cells pretreated with hyaluronidase, chondroitin sulfate, dextran sulfate and heparin failed to promote aggregation. Furthermore, chondroitin sulfate, heparin and dextran sulfate had no effect on cells induced to aggregate optimally by hyaluronan.
**L cells transfected with CD44Epican adhere to keratinocytes through an epican-mediated, hyaluronan-dependent mechanism**

The ability of epican to induce heterotypic cell-cell adhesion was tested by adding LEp-2H5 cells to monolayers of normal human keratinocytes. Adhesion of clone LEp-2H5 to keratinocytes was seven times greater than adhesion of the parent L cells and was uninfluenced by the calcium concentration (Fig. 7A). Adhesion of LEp-2H5 cells to keratinocyte monolayers was almost totally abolished by preincubation with the anti-epican antibody, mAb17 (Fig. 7B). Hyaluronidase pretreatment of keratinocytes and LEp-2H5 cells also prevented adhesion (Fig. 8), supporting a role for hyaluronan in epican-mediated adhesion. Most other glycosaminoglycans had only small effects on adhesion of LEp-2H5 cells to keratinocytes, but heparin at 1.5 µM blocked adhesion by more than 60% (Fig. 8).

**Epican can mediate keratinocyte-keratinocyte adhesion**

The ability of epican to mediate homotypic cell-cell adhesion between keratinocytes was tested under low calcium conditions chosen to minimize the strong adhesive action of keratinocyte cadherins. Adhesion of suspended keratinocytes to a confluent monolayer of keratinocytes was saturable (Fig. 9A). At low input (<10⁴ cells/well), more than 80% of the added keratinocytes adhered to the monolayer. At saturation density, approximately two cells adhered to each cell in the monolayer. For the adhesion experiments described below, 10⁶ cells were added to monolayers containing 2-3×10⁴ cells. Under those conditions, 15-30% of the suspended cells adhered to the monolayer.

The anti-epican monoclonal antibody, mAb17, inhibited keratinocyte-keratinocyte adhesion in a dose-dependent fashion (Fig. 9B). At 10 µg/ml, mAb17 blocked adhesion by more than 80%. H-3, the anti-CD44 monoclonal antibody that binds to all forms of CD44 on keratinocytes, also blocked adhesion but even at 50 µg/ml was no more effective than mAb17. Adhesion was uninfluenced by nonspecific IgG. The ability of mAb17 to block adhesion was uninfluenced by pretreatment with azide or cytochalasin D, indicating a direct effect of the antibody on the ligand-receptor interaction.

**DISCUSSION**

CD44H, the standard or hematopoietic form of CD44, mediates cell-cell adhesion by a mechanism that usually requires hyaluronan. The experimental results reported herein demonstrate that epican, the heparan/chondroitin sulfate proteoglycan that is the most prominent form of CD44 on keratinocytes, is also capable of mediating cell-cell adhesion by a hyaluronan-dependent mechanism. Despite the close relationship between CD44H and epican, the epican result could not have been readily predicted since: (a) there was a suggestion in the literature that alternatively spliced variants of CD44H lost their ability to bind hyaluronan and to cause cell adhesion; and (b) there was no precedent for proteoglycans functioning as cell-cell adhesion molecules. In addition to the molecular reasons for examining the function of epican, we wondered whether keratinocytes could use epican as a low avidity adhesion molecule to complement their high avidity cadherins.

Until recently, there was controversy regarding the ability of alternatively spliced variants of CD44 to bind hyaluronan and to cause cell adhesion. Shortly after CD44H was shown to bind hyaluronan and cause cell adhesion (Araffo et al., 1990; Miyake et al., 1990), CD44E, a splice variant that failed to cause cell adhesion and did not bind hyaluronan, was isolated from a human colon carcinoma cell line (Stamenkovic et al., 1991). By contrast, a murine homologue of CD44E retained its ability to cause adhesion and to bind hyaluronan (He et al., 1992). Most recently, a distinct human CD44E isolated from a leukemia cell line was fully capable of binding hyaluronan (Dougherty et al., 1994). The sequence of the colon carcinoma CD44E was found to differ by three amino acids from the leukemia CD44E. One of the amino acid substitutions was in the hyaluronan-binding region, which is common to all forms of CD44. Those data suggested that the inability of the colon cancer CD44E to bind hyaluronan resulted from a mutation in the N-terminal domain of CD44, rather than from the additional protein domains coded by the membrane-proximal alternative exons (Dougherty et al., 1994). Epican contains the...
with 10 µg/ml testes hyaluronidase (Hase) or in the presence of two concentrations of hyaluronan (HA), chondroitin sulfate (CS), heparan sulfate (HS), and heparin (Hep). Each bar is the mean ± s.e.m. of four assays.

Fig. 8. Effect of glycosaminoglycans on adhesion of epican-expressing cells to keratinocytes. Cells from the LEp-2H5 clone were added to monolayers of keratinocytes without pretreatment or additions (Con), following pretreatment with 10 µg/ml testes hyaluronidase (Hase) or in the presence of two concentrations of hyaluronan (HA), chondroitin sulfate (CS), heparan sulfate (HS), and heparin (Hep). Each bar is the mean ± s.d. of four assays.

amino-terminal hyaluronan-binding sequence (Kugelman et al., 1992) found in all forms except the colon carcinoma form of CD44, as well as the Lys158 and Arg162, which enhance HA-binding (Peach et al., 1993). The adhesion and aggregation data from the CD44E-p-transfected L cells demonstrate that epican, the largest known protein variant of CD44, retains its ability to bind hyaluronan and cause cell adhesion.

CD44E-p-transfected L cells acquired the ability to self-aggregate and to adhere to keratinocytes. In previously published studies exploring the mechanism of CD44-induced adhesion and aggregation, a role for hyaluronan was established by showing that adhesion or aggregation were inhibited by pretreatment of cells with hyaluronidase or by the addition of soluble hyaluronan (Miyake et al., 1990; Aruffo et al., 1990; Birch et al., 1991; Stamenkovic et al., 1991). In keeping with those studies, adhesion and aggregation of CD44E-p-transfected cells were blocked by hyaluronidase and restored by the readdition of hyaluronan. Taken together, our experimental results are consistent with a model in which hyaluronan acts as an adhesive bridge between two (or more) molecules of epican.

It has been reported that both epican (Haggerty et al., 1992) and CD44E (Brown et al., 1991) are expressed as heparan sulfate proteoglycans on keratinocytes, although chondroitin sulfate is the usual glycosaminoglycan added to CD44 core proteins (Jalkanen et al., 1988; Faassen et al., 1992). When CD44E was transfected into mouse L cells, chondroitin sulfate, not heparan sulfate, was covalently linked to the core protein (Stamenkovic et al., 1991), suggesting either that L cells could not add heparan sulfate to CD44 or that CD44E was not a heparan sulfate proteoglycan. A substantial portion of the epican expressed by mouse L cells transfected with CD44E is a heparan sulfate proteoglycan, indicating that L cells have the capacity to add heparan sulfate and that the epican core protein sequence is sufficient to direct the addition of heparan sulfate. Since CD44E-p-transfected L cells do not add heparan to CD44E, perhaps CD44E is not normally a heparan sulfate proteoglycan, and the heparan sulfate proteoglycan previously identified on keratinocytes as CD44E (Brown et al., 1991) may, in fact, have been epican.

Results of the glycanase digestion experiments indicate that epican is expressed on transfected cells either as a heparan sulfate proteoglycan, as a chondroitin sulfate proteoglycan, or without any glycosaminoglycan. Conditions that determine whether chondroitin or heparan sulfate will be added to a particular core protein have not been established, but at least one other proteoglycan, betaglycan, is expressed as an either/or, heparan/chondroitin sulfate proteoglycan (Cheifetz et al., 1988; Andres et al., 1989). It remains to be determined whether all post-translationally modified forms of epican are equally effective in binding hyaluronan and in causing cell adhesion.

Cell-cell adhesive functions of epican appear to be a direct result of its expression on the cell surface. While appropriate function of other forms of CD44 may require cell activation (Haynes et al., 1983; Arch et al., 1992), interaction with the actin cytoskeleton (Belitsos et al., 1990; Camp et al., 1991; Lokeshwar and Bourguignon, 1992) or binding of monoclonal antibodies (Shimizu et al., 1989; Belitsos et al., 1990; He et al., 1992), epican promotes adhesion at 4°C and in the presence of azide and cytochalasin D. Thus, neither normal cell metabolism, interaction with the actin cytoskeleton nor rearrangement of the cell membrane are necessary for its adhesive function.

Cell-cell adhesive mechanisms must be particularly complex in continuously renewing tissues. In the epidermis, for example, the adhesive molecules must maintain tissue integrity but also must allow movement of differentiating keratinocytes through the tissue and ultimately permit programmed tissue disintegration (desquamation). In the past, location alone was often the chief justification for hypothesizing that molecules found between keratinocytes were reasonable candidates for adhesion molecules. The studies described in this report provide experimental support for an old hypothesis: that acid mucopolysaccharides can serve as an intercellular cement in the epidermis.

There have been few experimental studies of keratinocyte-keratinocyte adhesion or aggregation. The adhesion and aggregation experiments reported herein identify a calcium-independent component of keratinocyte-keratinocyte adhesion. Surprisingly, more than 80% of this keratinocyte-keratinocyte adhesion was blocked by the anti-epican antibody, suggesting that epican, and not some other form of CD44, was primarily responsible for that adhesion. Indeed, antibodies that bind all forms of CD44,
including epican, never reduced adhesion more than mAb17 did, indicating that other forms of CD44 contribute minimally to adhesion in that assay. Thus, the antibody experiments establish a possible role for epican in keratinocyte adhesion.

The molecular analysis of the relative importance of epican in keratinocyte-keratinocyte adhesion is complicated by the presence of at least two other forms of CD44 and a variety of other potential adhesion molecules on keratinocytes. Members of the cadherin and integrin families of adhesion molecules are expressed on keratinocytes and are likely to be the important high avidity adhesion molecules. Cadherin-like molecules are found at all levels of the epidermis and are concentrated at the desmosomes (Buxton et al., 1993). Antibodies to desmoglein and the pemphigus vulgaris antigen cause blisters in the skin in vivo (Rock et al., 1990; Amagai et al., 1992) and in vitro (Schiltz and Michel, 1976), but there has been little direct experimental analysis of cadherin function in keratinocytes. Several integrins are found on basal keratinocytes (Larjava et al., 1990; Carter et al., 1990; Adams and Watt, 1991), and antibodies against β1 integrins disrupt cell-cell contacts between keratinocytes (Larjava et al., 1990) and inhibit keratinocyte-keratinocyte adhesion by 20-40% (Carter et al., 1990). Thus, while keratinocytes ultimately will be needed to understand the function of epican in its normal context, they might be too complicated for an initial analysis. Our strategy to transfect epican into a non-adhesive cell, allowed us to isolate the adhesive contribution of epican from the contributions made by other adhesive molecules and other forms of CD44 found on keratinocytes. Having shown that epican causes cell-cell adhesion through a hyaluronan-dependent mechanism, we must wonder why several hyaluronan-binding variants of CD44 are expressed on keratinocytes and how they function in relation to other keratinocyte adhesion molecules.

We thank Janet Fears for administrative assistance. This work was supported by a pilot project award from the Yale Skin Disease Research Center (NIH P30 AR41941 to J.R.B., L.C.K. and L.M.M.), by the Veterans Administration (L.M.M.) and by the Dermatology Foundation (J.G.H.).

REFERENCES


immunoglobulin fragments from endemic pemphigus foliaceous (1990). Monovalent Fab


Stamenkovic, I., Amiot, M., Pesando, J. M. and Seed, B. (1990). A lymphocyte molecule implicated in lymph node homing is a member of the hematopoietic and epithelial forms of CD44 are distinct poly-peptides with different adhesion potentials for hyaluronate-bearing cells. EMBO J. 10, 343-348.


(Received 13 May 1994 - Accepted 5 July 1994)