Previous studies established that uterine epithelial cells and cell lines express cell surface heparin/heparan sulfate (HP/HS)-binding proteins (Wilson, O., J acobs, A. L., Stewart, S., and Carson, D. D. (1990) J. Cell. Physiol. 143, 60–67; Raboudi, N., J ulian, J., Rohde, L. H., and Carson, D. D. (1992) J. Biol. Chem. 267, 11390–11393). The accompanying paper (Liu, S., Smith, S. E., Julian, J., Rohde, L. H., Karin, N. J., and Carson, D. D. (1996) J. Biol. Chem. 271, 11817–11823) describes the cloning of a full-length cDNA corresponding to a candidate cell surface HP/HS interacting protein, HIP, expressed by a variety of human epithelia. A synthetic peptide was synthesized corresponding to an amino acid sequence predicted from the cDNA sequence and used to prepare a rabbit polyclonal antibody. This antibody reacted with a protein with an apparent Mr of 24,000 by SDS-polyacrylamide gel electrophoresis that was highly enriched in the 100,000 × g particulate fraction of RL95 cells. This molecular weight is similar to that of the protein expressed by 3T3 cells transfected with HIP cDNA. HIP was solubilized from this particulate fraction with NaCl concentrations 0.8 M demonstrating a peripheral association consistent with the lack of a membrane spanning domain in the predicted cDNA sequence. HIP was not released by heparinase digestion suggesting that the association is not via membrane-bound HS proteoglycans. NaCl-solubilized HIP bound to heparin-agarose in physiological saline and eluted with NaCl concentrations of 0.75 M and above. Furthermore, incubation of 125I-HP with transfibots of the NaCl-solubilized HIP preparations separated by two-dimensional gel electrophoresis demonstrated direct binding of HP to HIP. Indirect immunofluorescence studies demonstrated that HIP is expressed on the surfaces of intact RL95 cells. Binding of HIP antibodies to RL95 cell surfaces at 4 °C was saturable and blocked by preincubation with the peptide antigen. Single cell suspensions of RL95 cells formed large aggregates when incubated with antibodies directed against HIP but not irrelevant antibodies. Finally, indirect immunofluorescence studies demonstrate that HIP is expressed in both luminal and glandular epithelium of normal human endometrium throughout the menstrual cycle. In addition, HIP expression increases in the predecidual cells of post-ovulatory day 13–15 stroma. Collectively, these data indicate that HIP is a membrane-associated HP-binding protein expressed on the surface of normal human uterine epithelium and uterine epithelial cell lines.

Heparan sulfate proteoglycans (HSPGs)1 located either on cell surfaces or in extracellular matrices are found in nearly all mammalian tissues (1–5). Functionally, HSPGs and a variety of HP/HS-binding proteins have been shown to participate in a diverse range of biological processes such as cell attachment, growth factor binding, cell proliferation, migration, morphogenesis, and viral pathogenicity (6–8). Several lines of evidence indicate that HSPGs play an important role during the initial attachment of the apical plasma membrane of trophodermal cells of the blastocyst to the apical plasma membrane of the uterine epithelium. In mice, HSPGs are expressed on the cell surfaces of two-cell stage and post-implantation stage embryos (9). Furthermore, blastocyst attachment to laminin, fibronectin, and isolated mouse uterine epithelial cells is inhibited by HP. Embryo attachment also is inhibited by the treatment of embryos with HP/HS lyases or inhibitors of proteoglycan biosynthesis (10, 11). Immunological studies of murine embryo implantation sites indicated that the core protein of the basement membrane HSPG, perlecan, and HP/HS chains are located between the apical cell surfaces of trophodermal cells and uterine epithelial cells during the peri-implantation stage (12). Expression of perlecan on the external trophodermal surface correlates with acquisition of attachment competence in vitro as well. Externally disposed H/HS-binding sites have been described on the cell surface of mouse uterine epithelial cells (13). Furthermore, using a heterologous cell adhesion assay, we demonstrated that HP/HS-like glycosaminoglycans participate in the initial attachment between two human cell lines, JAR and RL95, used to mimic the initial attachment of the human embryonic trophoderm to human uterine epithelial cells, respectively (14). As is the case for mouse uterine epithelia, the human uterine epithelial cell line, RL95, has specific, high affinity cell surface HP/HS-binding sites, which are sensitive to mild trypsin digestion of intact cells. Three tryptic peptides that retained HP/HS binding specificity were isolated from such trypsinates and partially amino-terminal sequenced (15). In the accompanying paper (36), the full-length cDNA sequence to one of these proteins, named HIP for HP/HS interacting protein, was obtained and shown to

1 The abbreviations used are: HSPGs, heparan/sulfate proteoglycans; BSA, bovine serum albumin; HP/HS, heparin/heparan sulfate; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis.
encode a cell surface protein with an M, of 24,000 when expressed in transfected 3T3 cells. HIP is expressed in a cell-type-specific fashion by many human cell lines, particularly those of epithelial origin.

In the current study, we have generated and characterized a rabbit antibody to a synthetic peptide designed from a predicted 16-amino acid sequence of HIP. These studies demonstrate that HIP is a peripheral membrane protein that directly binds HP and is expressed on the surfaces of normal human uterine epithelia and many uterine epithelial cell lines.

**EXPERIMENTAL PROCEDURES**

**Materials—Tissue culture media components were obtained from Irvine Scientific (Santa Ana, CA) and Life Technologies, Inc.**

**Urine 1**-Protein A was from ICN Radiochemicals (Irvine, CA). Tris, glycine, bovine serum albumin, urea, phenylmethlysulfonfyl fluoride, polyethylene, EMDTA, magnesium chloride, heparin, and hemoglobin were purchased from Sigma. Sodium dodecyl sulfate, β-mercaptoethanol, acrylamide, bisacrylamide, and Tween 20 were purchased from Bio-Rad. Sodium azide, as described above. RL95 cell surface components were radioiodinated for 10 min at 4°C. Samples of pellets were rinsed three times with PBS at 4°C and resuspended in homogenizing buffer (0.25 M sucrose, 5 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.25 mM diethiothreitol, and a mixture of protease inhibitors (10)) and homogenized on ice. The homogenate was centrifuged at 10,000 g for 10 min at 4°C. The 10,000 g supernatant was centrifuged at 100,000 g for 1 h. Supernatants were precipitated overnight at 4°C by the addition of trichloroacetic acid to a final concentration of 10% (v/v). Pellets were dissolved in 0.2 ml of sample extraction buffer and then precipitated and prepared for SDS-PAGE as described above.

**Heparin Aporase Affinity Chromatography—High speed (100,000 × g) membrane preparations were divided into equal parts and extracted either with 0.4 M NaCl in 5 mM Tris (pH 8.0) and centrifuged at 100,000 × g for 1.5 h. The 0.4 M NaCl-insoluble pellet was subsequently extracted with 0.8 M NaCl in 5 mM Tris (pH 8.0) at 4°C for 4 h and centrifuged 1.5 h at 100,000 × g.** The proteins eluted between 0.4 and 0.8 M NaCl was diluted to 0.15 M NaCl in 10% (w/v) trichloroacetic acid, acetone, p-aminosalicylic acid, calcium chloride, and sodium chloride were purchased from Fisher. Sodium chloride and methanol were purchased from EM Science (Gibbstown, NJ). Tissue culture plates (100 mm) were purchased from Falcon (Lincoln Park, NJ), and 24-well tissue culture plates were purchased to 0.5-ml pellet of prereduced heparin (Sigma) was fixed in 100% methanol for 10 min at room temperature, rehydrated in PBS at 4°C. Samples of pellets were rinsed 3 times with binding buffer at 4°C for 5 min and incubated with HRP antibody and binding subsequently visualized with a peroxidase ABC system using a diaminobenzidine substrate kit as described by the manufacturer’s instructions (Vector Labs, Burlingame, CA). A parallel gel run under exactly the same conditions was silver-stained as described (21) to visualize the migration positions of all proteins on the gel.

**Western blot analysis and immunocytochemical studies were conducted using polyclonal antibodies affinity purified with the synthetic peptide linked to maleimide-activated BSA (Pierce) conjugated to cyanogen bromide-activated-Sepharose (Sigma), using the manufacturer’s protocol.**

**SDS-Polyacrylamide Gel Electrophoresis and Western Blotting—Cells or particulate subcellular fractions were initially solubilized and then extracted with 0.4 M NaCl-insoluble pellet was subsequently extracted with 0.8 M NaCl in 5 mM Tris (pH 8.0) at 4°C for 4 h and centrifuged 1.5 h at 100,000 × g. The proteins eluted between 0.4 and 0.8 M NaCl was diluted to 0.15 M NaCl in 10% (w/v) trichloroacetic acid, acetone, p-aminosalicylic acid, calcium chloride, and sodium chloride were purchased from Fisher. Sodium chloride and methanol were purchased from EM Science (Gibbstown, NJ). Tissue culture plates (100 mm) were purchased from Falcon (Lincoln Park, NJ), and 24-well tissue culture plates were purchased to 0.5-ml pellet of prereduced heparin (Sigma) was fixed in 100% methanol for 10 min at room temperature, rehydrated in PBS at 4°C. Samples of pellets were rinsed 3 times with binding buffer at 4°C for 5 min and incubated with HRP antibody and binding subsequently visualized with a peroxidase ABC system using a diaminobenzidine substrate kit as described by the manufacturer’s instructions (Vector Labs, Burlingame, CA). A parallel gel run under exactly the same conditions was silver-stained as described (21) to visualize the migration positions of all proteins on the gel.

**Immunochemistry—Cells were grown on coverslips for 48 h in Dulbecco’s modified Eagle’s medium/Ham’s F12 (20) and transferred to nitrocellulose as described above for Western blotting. The unbound nitrocellulose was washed twice with methanol and incubated overnight with 3% bovine serum albumin (BSA) in PBS at 4°C. The plates were rinsed with PBS for 5 min at room temperature, and immediately used for staining. In all cases, the affinity purified H1P primary antibody was used at a concentration of 25 μg/ml and the secondary antibody, fluoro-Negated donkey anti-rabbit IgG (Amersham Corp.), at a 1:100 dilution. Rabbit antiserum to human factor VIII was used at a 1:30 dilution. Rabbit antiserum to mouse laminin was used at a 1:50 dilution. Anti-H1P to RL95 Cell Surfaces—RL95 cells were grown to 90% confluency in 24-well tissue culture plates with Dulbecco’s modified Eagle’s medium/Ham’s F12 containing 10% (v/v) fetal bovine serum. Cells were rinsed three times with Hank’s-buffered saline and reincubated for 15 min at 4°C in 0.5 ml of binding buffer (PBS containing 2 mM CaCl2, 2 mM MgCl2, 0.1% (w/v) hemoglobin, 1 mM NaN3, and 0.02% (w/v) NaN3). The binding buffer was removed, and 0.2 ml of binding buffer containing anti-H1P or nonimmune rabbit IgG was incubated for 45 min at 4°C in duplicate wells. 1G was added to the following concentrations, 0, 10, 50, 100, and 200 μg/ml. Cells were rinsed 3 times with binding buffer at 4°C for 5 min and incubated with binding buffer containing 125I-protein A (1 × 106 cpm/well) for 30 min at
Subcellular Distribution of HIP—An antibody was generated to a synthetic peptide sequence predicted from the full-length HIP cDNA sequence (36). The sequence was predicted to be hydrophilic and likely to be exposed on the external surface of the intact protein. The antibodies routinely used for the studies described below were affinity purified on a column composed of the intact protein. The antibodies routinely used for the studies described below were affinity purified on a column composed of the intact protein. The antibodies routinely used for the studies described below were affinity purified on a column composed of the intact protein. The antibodies routinely used for the studies described below were affinity purified on a column composed of the intact protein. The antibodies routinely used for the studies described below were affinity purified on a column composed of the intact protein.

Subcellular fractionation was used as an initial step to partially purify HIP for subsequent analytical studies. Fractionation of RL95 cells and subsequent Western blot analysis determined that HIP was most highly enriched in the 100,000 × g pellet; however, HIP was detected in other particulate fractions as well (Fig. 1). Lower molecular weight components immunologically related to HIP were detected in the 1000 × g/20 min and 10,000 × g/20 min particulate fraction. These components were presumed to be partially degraded forms of HIP. In contrast, HIP appeared to be quantitatively depleted in the 100,000 × g soluble fraction. A similar distribution of HIP was observed in JAR and HEC-1a cells, human trophoblastic and uterine adenocarcinoma cell lines, respectively (data not shown). The high speed particulate fraction was used further as the most convenient source of HIP.

NaCl Solubilization of HIP—The 100,000 × g particulate fraction was subjected to incubations with increasing concentrations of NaCl and then analyzed by Western blot analysis. At a NaCl concentration greater than 0.8 M, HIP was eluted from the membrane fraction into a 100,000 × g soluble fraction. A similar distribution of HIP was observed in JAR and HEC-1a cells, human trophoblastic and uterine adenocarcinoma cell lines, respectively (data not shown). The high speed particulate fraction was used further as the most convenient source of HIP.

Heparin Binding to HIP—The 0.8 M NaCl eluate from the 100,000 × g particulate fraction was diluted to 0.15 M NaCl and incubated with heparin-agarose (Fig. 4). Elution of the heparin-agarose was performed with increasing concentrations of NaCl demonstrated that HIP bound to heparin and was quantitatively eluted with NaCl concentrations of 0.8 M or greater. The solubilization of HIP with salt indicates that HIP is likely to be peripherally associated with the particulate fractions of cells. Conditioned media from RL95 cells were centrifuged at 100,000 × g, and the corresponding supernatant and pellet were analyzed by Western blot analysis (Fig. 3). HIP was not detected in secretions from RL95 cells, indicating that this protein is not secreted or released from RL95 cells to a significant extent.

Heparin Agarose—Heparin agarose with increasing concentrations of NaCl demonstrated that HIP bound to heparin and was quantitatively eluted with NaCl concentrations of 0.8 M or greater. The solubilization of HIP with salt indicates that HIP is likely to be peripherally associated with the particulate fractions of cells. Conditioned media from RL95 cells were centrifuged at 100,000 × g, and the corresponding supernatant and pellet were analyzed by Western blot analysis (Fig. 3). HIP was not detected in secretions from RL95 cells, indicating that this protein is not secreted or released from RL95 cells to a significant extent.

Subcellular Distribution of HIP—An antibody was generated to a synthetic peptide sequence predicted from the full-length HIP cDNA sequence (36). The sequence was predicted to be hydrophilic and likely to be exposed on the external surface of the intact protein. The antibodies routinely used for the studies described below were affinity purified on a column composed of the BSA-conjugated HIP peptide linked to agarose. As shown below, these antibodies reacted primarily with a protein with a molecular weight of 24,000 as estimated by SDS-PAGE and Western blotting. This molecular weight is similar to that observed for HIP in the particulate fraction of RL95 cells (Fig. 1). Lower molecular weight components immunologically related to HIP were detected in the 1000 × g/20 min and 10,000 × g/20 min particulate fraction. These components were presumed to be partially degraded forms of HIP. In contrast, HIP appeared to be quantitatively depleted in the 100,000 × g soluble fraction. A similar distribution of HIP was observed in JAR and HEC-1a cells, human trophoblastic and uterine adenocarcinoma cell lines, respectively (data not shown). The high speed particulate fraction was used further as the most convenient source of HIP.
HIP is not secreted or released from RL95 cells. Serum-free RL95 cell-conditioned medium from a 24-h incubation was collected and centrifuged at 100,000 x g for 1 h. The 100,000 x g supernatant was trichloroacetic acid-precipitated, and equal portions of all fractions were analyzed for the presence of HIP by Western blotting as described under "Experimental Procedures." Lane 1, RL95 cell homogenate; lane 2, 100,000 x g pellet from conditioned medium; lane 3, the 100,000 x g supernatant from conditioned medium.

HIP binds tightly to heparin-agarose. The 0.8 M NaCl extract of a 100,000 x g60-min particulate fraction was subjected to heparin-agarose chromatography as described under "Experimental Procedures." A portion of the sample was used for direct Western blot analyses (Ext.), and the remainder was diluted to 0.15 M NaCl before incubation with heparin-agarose. HIP was serially eluted batchwise from heparin-agarose with buffers containing increasing concentrations of NaCl as indicated at the top of the figure. Each eluate was trichloroacetic acid-precipitated and analyzed by Western blot analyses. Each lane (0.15–2.0 M NaCl) represents the total material obtained in each eluate.

Fig. 5, many proteins co-isolated with HIP by this procedure (panel A); however, only a subset of these proteins retained the ability to bind 125I-HP (panel B). Collectively, the binding and elution of HIP from heparin-agarose and the coincident binding of 125I-HP and anti-HIP indicate that HIP is a HP-binding protein.

Cell Surface Localization of HIP—Anti-HIP was used to determine if this protein was expressed on the external surface of intact cells. Initially, concentration dependence and saturability of anti-HIP binding was examined. Fig. 6A shows that binding of anti-HIP to intact RL95 cells was both specific and saturable as compared with binding of nonimmune rabbit IgG. Furthermore, when anti-HIP protein was pre-absorbed with peptide affinity matrix, its binding was reduced to the level of nonimmune rabbit IgG (Fig. 6B). Next, anti-HIP was used to examine the distribution of this protein on HEC-1a cell surfaces. As shown in Fig. 7, immunostaining of methanol-permeabilized, paraformaldehyde-fixed HEC-1a cells with anti-cytokeratins demonstrated a strong positive signal (panel B). In contrast, fixed, nonpermeabilized cells displayed only background staining (panel A) comparable with that observed when primary antibody was omitted (panel D). Staining of fixed, nonpermeabilized cells with anti-HIP was uniformly distributed on the surfaces of all cells in these cultures including points of cell-cell contact (panel C). Similar results were obtained with RL95 cells (data not shown). Collectively, these data indicated that reactivity with anti-HIP was reflective of cell surface staining and not due to permeabilization in human uterine epithelial cell lines.

It was further reasoned that if HIP was on RL95 cell surfaces then non-fixed, single cell suspensions of living RL95 cells could be aggregated by anti-HIP. As shown in Fig. 8, incubation of single cell suspensions of RL95 cells with anti-HIP greatly enhanced cell aggregation. Parallel controls, including PBS, PBS containing 0.02% sodium azide and an antibody to the cytoplasmic tail of the mucin, MUC1 (24), did not enhance RL95 cell-cell aggregation. Collectively, these data strongly indicate that HIP is located on the extracellular surface of the plasma membrane of human uterine epithelial cell lines.

Experiments also were performed to determine if HIP is expressed by other human uterine epithelial cell lines as well as normal human uterine epithelium in situ. As shown in Fig. 9, Western blots of several human uterine epithelial cell lines as well as human endometrium displayed a prominent band corresponding to the molecular weight of HIP. A 1.3-kilobase transcript is detected in all three cell lines by Northern analyses using HIP cDNA as a probe (36). HIP Expression in Human Endometrium—Expression and localization of HIP was examined in methanol-fixed frozen
sections of human endometrium taken at various stages throughout the menstrual cycle. In all cases, strong reactivity of lumenal and glandular epithelia was detected. Through the proliferative and until post-ovulatory day 7 of the cycle, HIP reactivity was not detected in underlying stroma cells (Fig. 10). Nonimmune IgG failed to react with these tissues (data not shown). Furthermore, the epithelial identity of the HIP-positive cells was confirmed by demonstration of reactivity with antisera to cytokeratins and Muc-1 in serial sections (data not shown). Strong reactivity was detected at both the apical and basal aspects of these cells. Some variation in the intensity of signal between these glandular structures was noted. It is unclear if this variation reflects differences between glands or regional differences in HIP expression of individual glands that normally extend from the uterine lumen (functionalis) to deep within the endometrium (basalis). By post-ovulatory day 13, additional staining for HIP was detected within the underlying stroma (Fig. 11). As expected, the underlying stroma extracellular matrix also displayed strong expression of the decidual marker, laminin (27), at this time. In contrast, laminin expression was confined to basal lamina in stromal tissue of late proliferative stage uteri. The heparan sulfate proteoglycan, perlecanc, also has been reported to be expressed by decidualizing stroma cells (26); however, stromal staining for perlecanc was much less intense than that of basal lamina (data not shown). As mentioned above, HIP was not detected in stromal cells through the entire proliferative phase of the cycle. These data demonstrated that HIP is a protein normally expressed by uterine epithelia.
Uterine Heparin/ Heparan Sulfate-binding Protein

A number of studies described above have demonstrated that HSPGs are expressed on the surfaces of mouse blastocysts and human trophoblastic cell lines where they function in cell adhesion events. In these studies, it was further demonstrated that adhesive activity resides in the constituent HS chains of the HSPGs. Consistent with these observations, specific HP/HS-binding sites were identified on the surfaces of both mouse uterine epithelial cells and human uterine epithelial cell lines (13, 15). HP/HS-binding sites have been described on the surfaces of a number of cell lines (28–31); however, identification of these proteins has been elusive. N-CAM represents one well described cell surface HP/HS-binding protein (32) but is not expressed in the uterus. Recently, heparin-binding epidermal growth factor-like growth factor was identified at mouse implantation sites (33) and is one potential ligand for embryonic HSPGs. Several other candidate proteins have been described that display HP/HS-binding activity (34, 35) but have not been well characterized. In previous studies, we were able to obtain a partial amino-terminal sequence of several tryptic peptides derived from RL95 cell surfaces that retained HP/HS-binding activity. This sequence was used to obtain a full-length cDNA and predicted amino acid sequence of one of these proteins (36). This protein is referred to as HIP. Inspection of the predicted amino acid sequence of HIP using several protein structure-predicting algorithms indicated regions likely to be antigenic and exposed on the exterior surface of the protein. One of these sequences was chosen for preparation of antibodies, and these antisera have been used in the present study.

The predicted pI of HIP, >10, is consistent with its behavior on isoelectric focusing gels. Alternatively, HIP may be posttranslationally modified. No consensus sites for glycosylation are indicated by the predicted sequence; however, other modifications are possible. Subcellular fractionation studies indicate that HIP is most highly enriched in the high speed particulate fraction and is quantitatively depleted from the high speed supernatant, i.e. cytosolic fraction. We have detected various plasma membrane markers in this fraction including 

**DISCUSSION**

Na\(^{+}\)/K\(^{-}\)-ATPase and radiiodinated cell surface components\(^2\); however, rearrangement of peripheral membrane components like HIP may occur during such fractionation making interpretation of subcellular locale by this approach problematic. The ability of NaCl to release HIP from the particulate fraction is consistent with the lack of a potential membrane spanning

\(^2\) J. Julian and D. D. Carson, unpublished studies.
domain in the predicted sequence of HIP and demonstrates that HIP is a peripheral membrane protein. Digestion of membranes with a mixture of HP/HS lyases did not release HIP into the 100,000 × g soluble fraction. This suggests that HIP is not retained by membrane-bound HSPGs. Therefore, it is possible that other membrane components bind and retain HIP. Alternatively, it is possible that HIP binds to a region of HS close to the protein core and protects HS from enzymatic digestion. Characterization of the HIP-binding sites is necessary to define the nature of the HIP-membrane interaction.

Several lines of evidence indicate that HIP is displayed on cell surfaces. Antibodies to this protein bind specifically and in a saturable manner to intact RL95 cells under conditions where endocytosis should not occur. Assuming a 1:1 stoichiometry of IgG binding to HIP and protein A to antibody, it can be calculated that there is an average of approximately 1.5 × 10^4 molecules of HIP displayed on the surface of each RL95 cell. If each IgG binds to two HIP molecules and each protein A tetramer binds four IgG molecules then this estimate may be as high as 1.2 × 10^5 HIP molecules per cell surface. In either case, these numbers are well below the number of [^3H]HIP-binding sites (9 × 10^6) previously determined for RL95 cells (15). Consequently, even given potential inaccuracies in both estimates, it seems that HIP can only be one of multiple cell surface HP/HS-binding proteins displayed on RL95 cell surfaces. It is possible that many HIP molecules are occupied by HS at the cell surface and masked from antibody binding. HS lyase pretreatment of cells did not expose additional anti-HIP-binding sites; however, if, as discussed above, HIP binding “protects” HS chains from digestion then HS lyases might not be expected to expose more HIP.

Antibodies to HIP also display staining patterns on intact RL95 cells that are consistent with those of cell surface components, e.g. enrichment at cell peripheries and regions of cell-cell contact. Similar patterns of immunoreactivity with anti-HIP are detected on human trophoblastic and breast cancer cell lines. Furthermore, these same antibodies specifically aggregate RL95 cells in suspension, a property expected for antibodies reacting with epitopes displayed on the cell surface. Experiments with an impermeant chemical cross-linking reagent destroyed antibody reactivity with HIP, but larger cell-associated bands were not observed. Thus, while in one sense these experiments suggest a cell surface disposition of the protein, the apparent destruction of the epitope confuse interpretation. Collectively, these data strongly argue that at least a fraction of the population of HIP is displayed on RL95 cell surfaces where these proteins may directly participate in HP/HS binding.

HIP is detected in several human uterine epithelial cell lines and in human endometrium by Western blotting of total protein extracts. Moreover, anti-HIP strongly reacts with uterine epithelial cells in sections of human endometrium through post-ovulatory day 7 of the cycle. By post-ovulatory day 13, HIP is also detected in the predecidual cells of the uterine stroma. The HSPG, perlecan, is expressed by human decidual cells (26). It is possible that HS chains of perlecan also serve as ligands for HIP in basal lamina and in the decidial extracellular matrix. In any event, these observations indicate that HIP is expressed by normal human endometrium. Potential functions could involve binding to basal lamina or intercellular HSPGs expressed by uterine epithelia or HSPGs expressed by blastocysts during implantation. The antibody described in the present studies does not react with mouse uterine components either by immunostaining or Western blotting. Current efforts are being placed toward generating probes to the mouse homologue so that the physiological role of this protein in the uterus can be more rigorously examined by molecular genetic approaches.

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