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Differential Expression of CD44 in Rabbit Uterine Epithelium during Early Pregnancy

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Abstract
The expression of the cell surface glycoprotein CD44 was monitored in rabbit endometrium during early pregnancy and pseudopregnancy by immunohistochemistry. The epitope was not detected in the uterine epithelium of nonpregnant does; in pseudopregnant animals it was expressed only weakly and late, most clearly detectable at the last stage investigated, i.e. on day 10. During pregnancy, however, CD44 was expressed more strongly in the epithelium starting on day 6, i.e. shortly before embryo implantation (day 7). Northern blot analysis confirmed this increase in expression. Immunohistochemically, CD44 expression peaked around days 8 and 9 of pregnancy and was generally localized on the lateral cell membranes of uterine epithelium, but not on basal or apical membranes. The staining pattern was similar on all major mucosal folds in that the signal was most intense in the luminalmost parts and slightly less in the middle of these folds. The intensity was gradually reduced towards the depth of the crypts with their deepest parts being negative. At day 10 of pregnancy the intensity of staining was clearly reduced in all parts of the epithelium that had been positive before. Fusion of epithelial cells, a characteristic phenomenon in pregnant rabbit uteri, which is particularly widespread in the implantation chamber, was accompanied with abolishment of CD44 expression. While stromal cells in general showed only a weak reaction, some individual cells in the stroma were always strongly positive (numbers increased after implantation). The trophoblast only occasionally exhibited some faint cellular staining in cytotrophoblast as well as in syncytiotrophoblast. These data show that CD44 is expressed in rabbit uterine epithelium during the periimplantation phase, and that its expression appears to be triggered by embryonic signalling and may be relevant for implantation.

Introduction

The uterine epithelium appears to play a critical role in controlling trophoblast adhesion and invasion in the process of embryo implantation. The hormone-dependent establishment of endometrial 'receptivity' [Psychoyos, 1976] is associated with a complex set of morphological and biochemical transitions of the uterine wall, in particular of the uterine epithelium. Changes observed in the periimplantation phase are thought to prepare the epithelium to permit trophoblast to adhere and to penetrate, a remarkable phenomenon in a polarized epithelium that should be nonadhe-

Abbreviations used in this paper:
BSA = Bovine serum albumin; d p.c. = days post coitum; d p. hCG = days after injection of hCG; FITC = fluorescein iso-thiocyanate; hCG = human chorionic gonadotropin; IU = international units; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate; SSC = sodium chloride/sodium citrate solution.

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sive for other cells. Modulations of molecular markers have been reviewed recently by Denker [1993] and include alterations of the glycocalyx, cell surface charge, cellular junctions with related molecules, the expression of new membrane molecules, and membrane enzymes associated with cell polarity. However, for most of these markers a functional significance could not yet be demonstrated. The most specific suggestions concerning involved molecules that may be expressed differentially refer to heparan-proteoglycans and corresponding binding molecules [Carson et al., 1993; Raboudi et al., 1992; Rohde and Carson, 1993]. Another possible pair of adhesion molecules proposed to play a role here are glycosyltransferases and the appropriate cell surface proteoglycans or glycoproteins [Chávez, 1990]. Differential expression of integrins in the uterine epithelium has been observed during the menstrual cycle in the human [Lessey et al., 1992] and may reflect only differential changes in cell-cell or cell-substrate adhesion. Their proposed significance for trophoblast adhesion, however, awaits experimental proof since little is known about possible ligands for these receptors on the trophoblast [Carson et al., 1990].

CD44 is an integral cell membrane proteoglycan which is involved in cell-cell and also in cell-matrix interaction depending on the isoform expressed [Underhill, 1992]. This molecule is expressed on a wide variety of cells with certain isoforms being found on a subset of epithelia including the uterine epithelium while others are detected in hematopoetic cells or fibroblasts [Flanagan et al., 1989; Stamenkovic et al., 1989; Brown et al., 1991; Stamenkovic et al., 1991; Mackay et al., 1994]. In epithelia, the expression of CD44 is correlated with the state of differentiation although its role as cell adhesion or matrix adhesion molecule still seems to be unclear as is its regulation. On tumor cells, isoforms of the molecule have been implicated to facilitate metastasis by mediating adhesion of circulating cells to vascular endothelium [Günthert et al., 1991]. These observations evoke speculations that in the uterine epithelium CD44 expression could be correlated with the hormone-dependent processes of differentiation and receptivity and may be involved in trophoblast adhesion. However, the possibility of differential epithelial expression of this molecule during the menstrual cycle or in different phases of pregnancy has not been investigated so far.

In this report we show that CD44 becomes expressed in rabbit uterine epithelium in the periimplantation phase of pregnancy whereas only little is found in pseudopregnancy suggesting a regulation in this tissue by embryonic signals in addition to maternal steroid hormones, implying that embryonic signalling molecules and appropriate uterine receptors are an important pathway of CD44 regulation in rabbit uterine epithelium. Since the molecule is found on lateral membrane domains CD44 may be involved in changes of cell-cell interactions rather than in altered cell-matrix interactions.

Materials and Methods

Animals

Sexually mature female rabbits (mixed breeds) were caged individually in air-conditioned quarters under a light-dark cycle of 12/12 h and fed a standardized pellet diet ad libitum. Pseudopregnancy was induced by a single i.v. injection of 75 IU human chorionic gonadotropin (hCG, Prolan®, Bayer, Leverkusen, Germany). Pseudopregnancy is an established model for conditioning of the uterus by maternal steroid hormones without any contribution of factors locally delivered by blastocysts [Beier and Kühnel, 1973]. Other females were mated with two bucks of proven fertility. The day of mating and the day of hCG injection were designated as day 0. At defined stages of pregnancy (3, 5, 6, 7, 8, 9, and 10 d p.c. = days post coitum) or of pseudopregnancy (d p. hCG = days after injection of hCG) at least 2 does on each day were euthanized by stunning and exsanguination. Two nonmated, noninjected females were included for comparison. The uterus was removed quickly and cut into small segments. In pregnant animals, the implantation chambers and blastocyst-free segments were separated. This material was frozen unfixed in liquid nitrogen and stored at −25 to −30 °C in air-tight plastic bags.

Immunohistochemistry

An affinity-purified mouse monoclonal antibody recognizing the standard 85- to 95-kD isoform of the transmembrane molecule CD44 [Pesando et al., 1986; Stamenkovic et al., 1989] was obtained from Immunotech S.A. (Marseille, France). Polyclonal goat anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) was purchased from Dako GmbH (Hamburg, Germany) as well as goat nonimmune complete immunoglobulin fraction.

Immunohistochemical staining was carried out on 10-µm-thick cryostat cross-sections which were mounted on glass coverslips, air-dried, fixed in ice-cold methanol for 5 min, rehydrated and washed with PBS (phosphate-buffered saline), and then preincubated for 2 h with goat nonimmune serum diluted 1:50 in PBS containing 1% bovine serum albumin (BSA, Sigma, Deisenhofen, Germany). The sections were incubated with the first antibody (anti-CD44 diluted 1:10 in PBS/BSA) for 2 h at room temperature in a humid atmosphere. After 3 rinses with PBS, the FITC-conjugated second antibody (diluted 1:100 in PBS/BSA) was applied for 2 h at room temperature in a humid atmosphere in the dark. After 3 rinses with PBS the sections were mounted on glass slides with glycerol containing 0.1% *p*-phenylenediamine to retard photobleaching. Then they were studied and photographed with a Zeiss Axiophot microscope equipped for epifluorescence. Control sections were treated with either of the antibodies alone (after incubation with nonimmune serum). In all cases this resulted in no or very faint cytoplasmic or nuclear staining. The same standardized exposure times were used for all immunofluorescence micrographs. For rabbit-specific terminology defining uterine regions described in this publications, see figure 1.

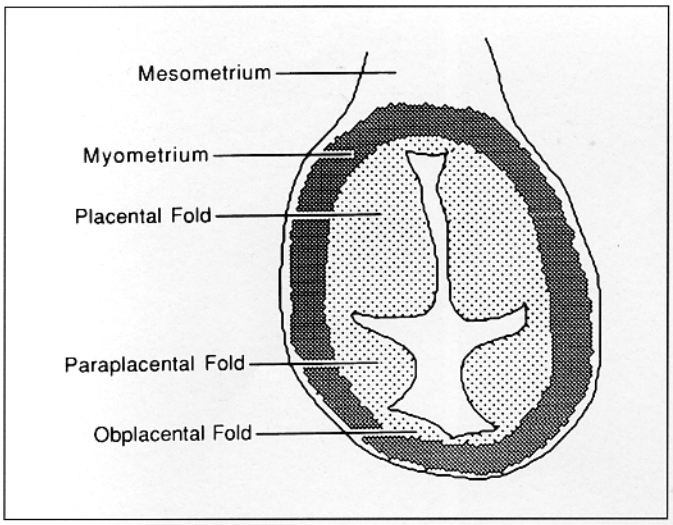


Fig. 1. Schematic view of uterine structures in the nonpregnant rabbit (cross-section).

Northern Blot Analysis

For Northern blot analysis endometrium was separated from the myometrium and frozen in liquid nitrogen immediately after explantation of the uterus. Rabbit lung tissue was taken as a control. Total cellular RNA was isolated by the guanidinium isothiocyanate/cesium-chloride method [Sambrook et al., 1989] and quantified spectrophotometrically at 260 nm. In order to estimate the purity of RNA samples, the ratio of optical densities at 260 and 280 nm was determined and was adjusted to about 1.6–1.8 in all preparations. RNA samples were separated electrophoretically on 1.2% agarose gels containing 2.2 *M* formaldehyde. The amounts of ribosomal RNA in each lane were visually compared after staining the gels with ethidium bromide. The RNA was blotted onto nylon membranes (Hybond-N, Amersham-Buchler GmbH, Braunschweig, Germany) and incubated at 80 °C for 2 h. Isolated restriction fragments of plasmid DNA were labelled with $\alpha[^{32}P]$dCTP to a specific activity of 2 to 5×10^8 cpm/µg using Klenow DNA polymerase (Boehringer, Mannheim, Germany) and random oligonucleotides as primers (kit from Amersham-Buchler) [Feinberg and Vogelstein, 1983]. After prehybridization at 42 °C for 3 h in a solution containing 55% deionized formamide, 1 *M* NaCl, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate (500 kD), and 100 µg/ml salmon sperm DNA, hybridization was carried out with 25 ng labelled DNA at 42 °C for 12–16 h using the same buffer.

For hybridization a cDNA of 350 bp corresponding to the 5' constant coding region of human CD44 (pos. 385–744 [Stamenkovic et al., 1989]) was kindly provided by P. Herrlich (Karlsruhe, Germany). Blots with specific probes were washed consecutively in 2×SSC (0.15 *M* NaCl plus 0.015 *M* sodium citrate) containing 1% SDS at 60 °C, in 1×SSC with 0.1% SDS at 60 °C for 1 h, and in 0.5×SSC/0.1% SDS for 0.5 h. The membranes were exposed to Kodak XAR-5 films at −80 °C with intensifying screens. For consecutive hybridization, the gene probe was removed from the membrane by immersing the membranes in boiling water and cooling down to room temperature. Before a new hybridization was started, the membrane was autoradiographed to check that the previous probe had been removed completely.

Results

Nonpregnant and Pseudopregnant Rabbits

CD44 immunohistochemistry of uteri from nonpregnant and pseudopregnant does generally resulted in a weak-to-moderate reaction of smooth muscle cell borders in the myometrium and in the vasculature (fig. 2). Stromal cells exhibited only a weak signal on their cell boundaries except for a subpopulation of scattered cells with a very strong reaction on their plasma membranes. These cells, probably representing leukocytes, were randomly located in all stromal areas without significant differences in frequency between the different stages of pseudopregnancy and the nonpregnant state. The uterine epithelium was negative until day 3 p. hCG, but later on exhibited some faint staining of the cell membranes of mononuclear cells and of symplasms. At these early stages the low intensity of staining did not allow to judge clearly whether this signal was distributed evenly around the entire circumference of the cells, as it appeared, or whether there was a slight lateral predominance. From 7 d p. hCG on, patches of staining slightly above that weak signal occurred occasionally in the upper parts of the crypts. A clear but still weak-to-intermediate reaction of the lateral membrane domains of epithelial cells was observed in a patchy distribution over all mucosal folds on day 10 of pseudopregnancy (fig. 3; table 1). This signal was usually pronounced in the luminal epithelium and the upper parts of the crypts with a decreasing gradient down to the bottom of glandular tubes (which were always negative).

Pregnancy

The *uterine epithelium* did not display reactivity until day 6 p.c. except for the same faint signal on cell membranes that was also seen during pseudopregnancy, again starting at day 3 p.c. At 7 d p.c., the epithelium of the *blastocyst-free segments* had developed a reactivity that was slightly increased above the level of pseudopregnancy, with still heterogeneous distribution (fig. 4b). This staining was found in the luminal and uppermost parts of the crypts located at the tips of the major mucosal folds, and it decreased on the flanks of the major folds. Some of the crypts at the base of the mucosal folds had no signal. On day 8 p.c., the CD44 immunofluorescence had increased further and comprised all major mucosal folds (fig. 5a). It remained constant until 9 d p.c., but was again diminished to moderate staining at 10 d p.c. (table 1). At maximal expression on days 8 and 9 p.c., luminal epithelium and the upper third of endometrial crypts were equally strongly positive, while the signal decreased in the lower third

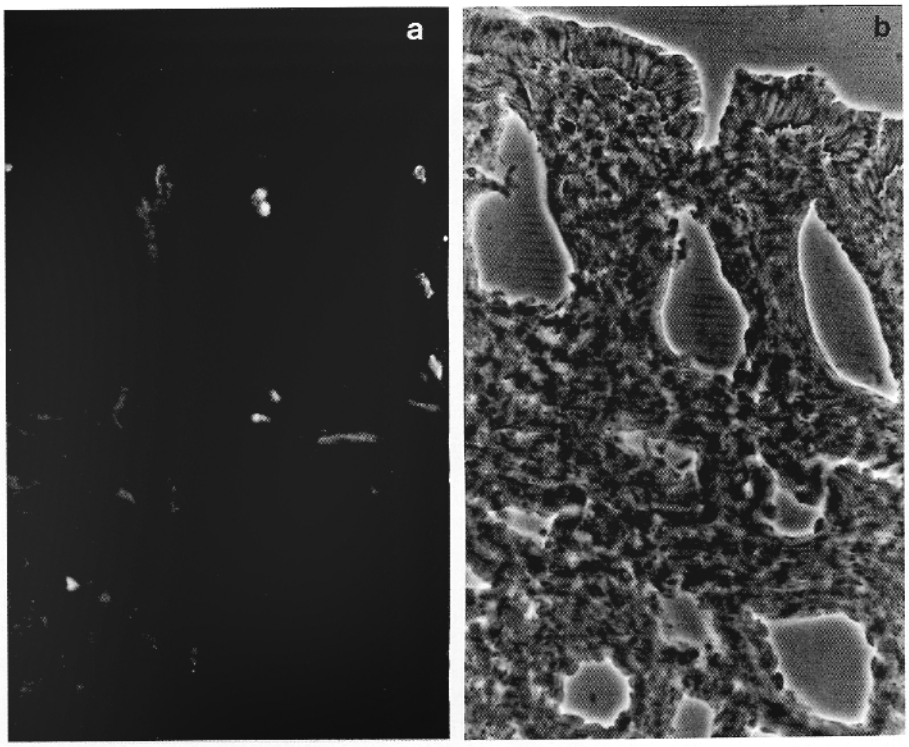


Fig. 2. **a** Absence of CD44 immunofluorescence staining in nonpregnant rabbit uterine epithelium. Stromal elements exhibit weak staining except for a small subpopulation displaying a stronger signal. **b** Phase-contrast image. × 340.

Table 1. CD44 immunofluorescence intensity in uterine epithelium

	0 days	3/5 days	6 days	7 days	8 days	9 days	10 days
Pseudopregnancy	–	(+)	(+)	(+)/+	(+)/+	(+)/+	(+)/+/++
Pregnancy							
Blastocyst-free segments		(+)	(+)	+/++	+++	+++	++
Implantation chamber							
Individual cells and small symplasms		(+)	(+)/+	++	+++	+++	++/+++
Large symplasms					–[1]	–[1]	–[1]

Staining intensity: – = No staining; (+) = very weak; + = weak; ++ = intermediate; +++ = strong.

[1] Weak perinuclear staining was observed in symplasms of up to intermediate size.

resulting in no or only faint reactivity in the deepest parts of the crypts. On day 10, CD44 expression had returned to moderate intensity similar to that observed on day 7, but staining was more homogeneous. The reactivity was comparable in all the major mucosal folds with just the areas between them staining a little weaker.

In all stages, the immunofluorescence signal was located on the lateral but not on the apical or basal domains of the cell membrane. Differences in this membrane distribution of CD44 between individual cells and symplasms could not be established (fig. 5). Progressing cell fusion was associated with overall reduced staining, particularly in placental and obplacental folds, although cell membranes were always positive. On the other hand, while mononuclear epithelial cells were reactive only on the cell membranes, weak additional cytoplasmic staining was noticed in symplasms where it appeared as a perinuclear haze (fig. 6).

Blastocyst-containing segments could not be identified before 6 days of pregnancy and all samples displayed the same behavior until then. However, while in interblastocyst regions significant expression was not detected before day 7, in blastocyst-containing segments it was observed first

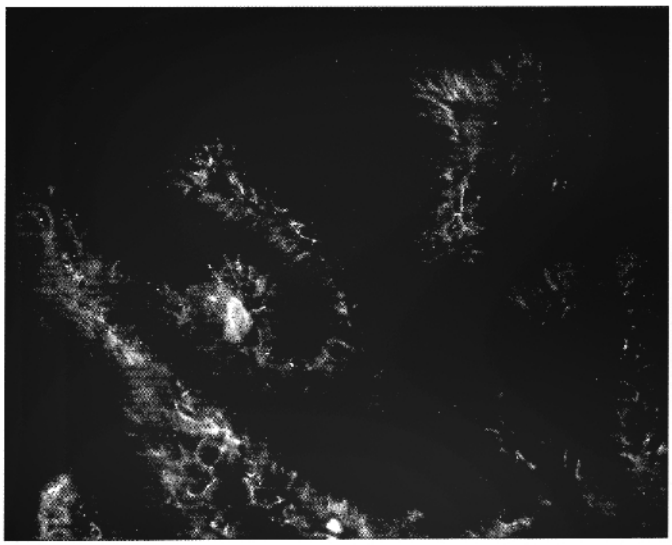


Fig. 3. CD44 immunofluorescence in pseudopregnancy, day 10. A very low but positive signal is obtained on lateral plasma membranes of epithelial cells, varying between cells. ×350.

on day 6. At that time, CD44 expression appeared to be variable but in some limited patches it was increased slightly above the faint membrane stain seen in interblastocyst parts before day 7 p.c. (fig. 4a).

During the following days of pregnancy, the time course of CD44 expression in blastocyst-containing segments as well as the cellular distribution was basically comparable to that described for the intersegments, with the exception of the large symplasmic masses (homokaryons and heterokaryons) that only form at implantation sites (table 1). In these areas of massive fusion of epithelial cells in the placental and obplacental folds, these symplasmic masses were found free of CD44 immunofluorescence (fig. 7, 8). Cellular epithelium remaining in the deeper crypts underneath such areas exhibited a clear weak-to-moderate signal with transition to negative cells in the bottom of the glands (fig. 8, 9). In areas of prospective cell fusion and in small symplasms, the lateral signal appeared to be reduced first in the apical part of the lateral membrane domains before it was lost completely, i.e. it seemed to be shifted transiently to more basal domains of the lateral membrane. In those parts of the epithelium of the placental folds which had not been transformed into large symplasmic masses labelling

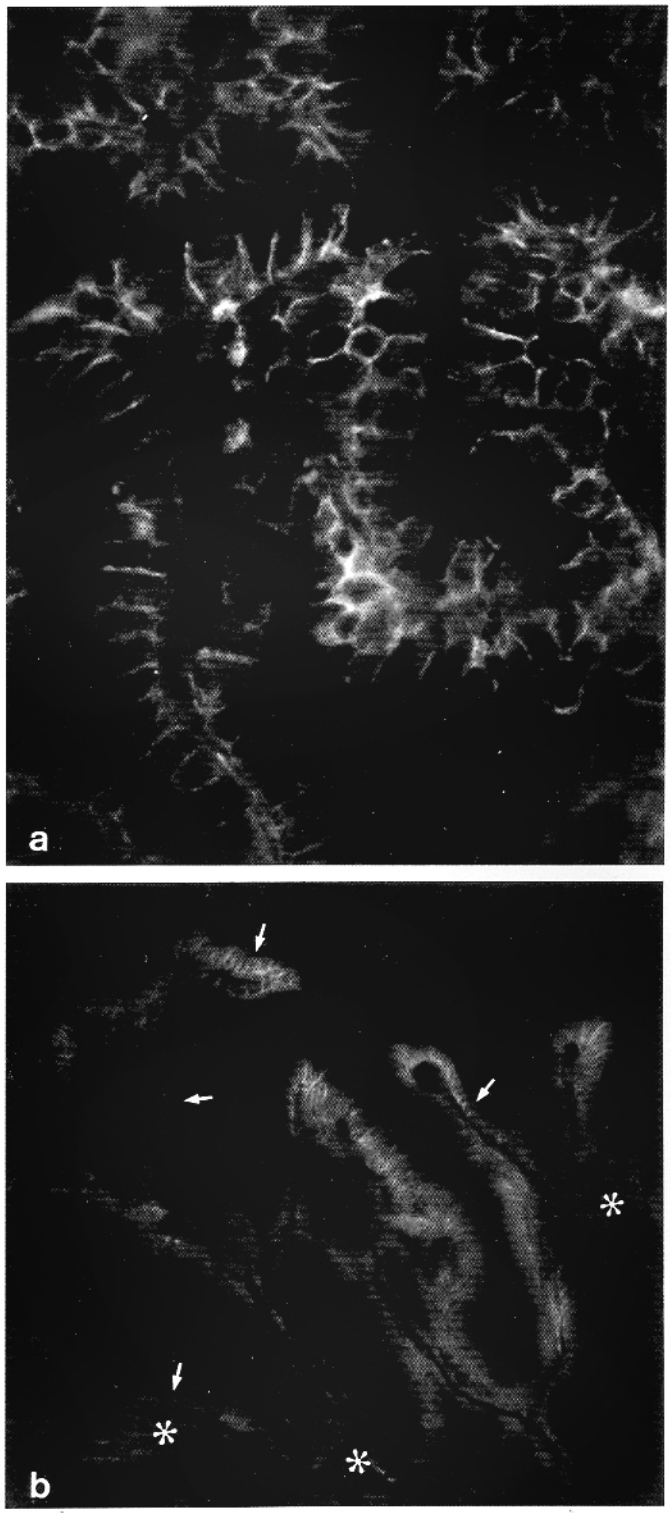


Fig. 4. In pregnancy, CD44 is detected first in blastocyst-containing segments on day 6 (**a**). In intersegments, a clear signal is not obtained before day 7 (**b**). The weak signal observed in the implantation chamber on day 6 of pregnancy (**a**) is located on the lateral membranes in patches of mononuclear and oligonuclear cells, in the vicinity of the blastocyst as well as in more distant areas. The interblastocyst segment on 7 d p.c. (**b**, lower magnification) shows a very heterogeneous distribution of epithelial cells staining weakly to moderately on the lateral membranes (arrows). Stromal elements (asterisks) exhibit only faint staining. **a** ×400. **b** ×200.

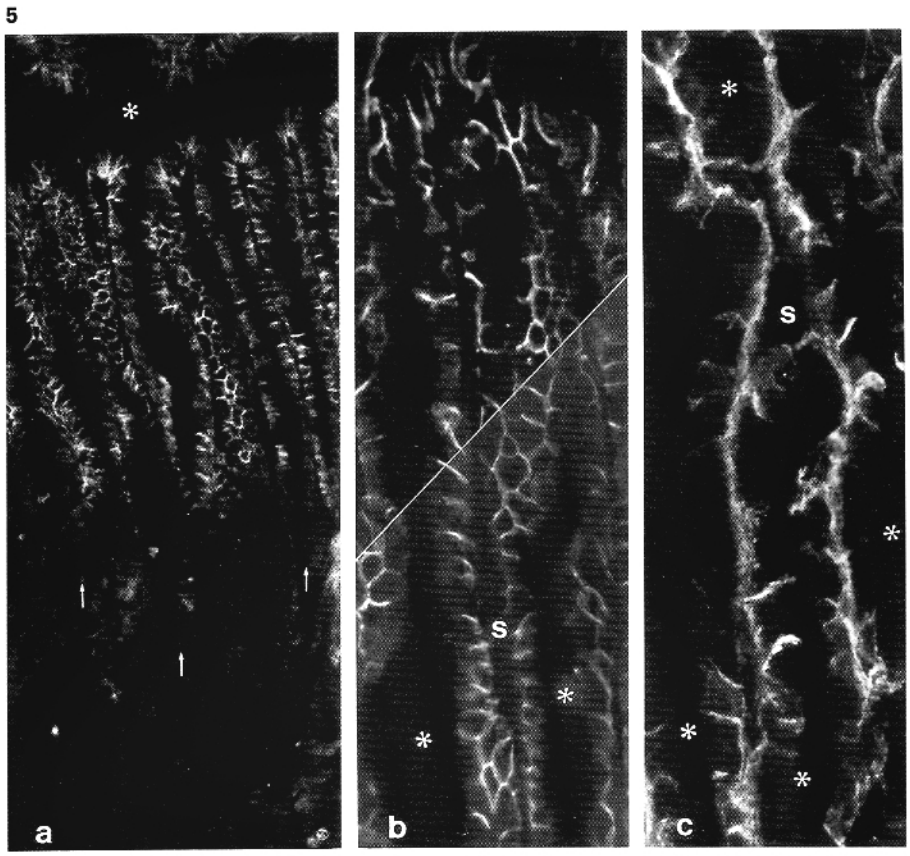


Fig. 5. Maximal expression of CD44 immunofluorescence in rabbit uterine epithelium. **a** Blastocyst-free segment on 9 d p.c.; detail from the flank of the paraplacental fold depicting the homogeneous reactivity on the lateral membranes of mononuclear and oligonuclear epithelial cells in the upper regions of the crypts; deeper parts of the crypts are negative (arrows). **b** Higher magnification from the upper third of crypts on the placental fold of an interblastocyst segment on 8 d p.c. demonstrating clearly that the signal is localized on the lateral but not on apical or basal cell membranes. **c** 9 d p.c., implantation chamber, placental fold. Proceeding cell fusion is associated with a shift of signal to the basal parts of the basolateral membrane in contact with the basement membrane. Asterisks = Lumen; s = stroma. **a** ×175. **b**, **c** ×350.

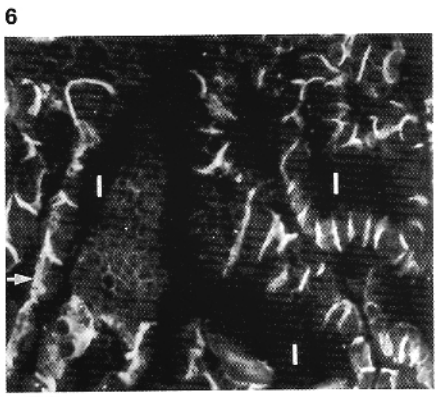


Fig. 6. Localization of CD44 in different stages of symplasm formation in interblastocyst segments at 8 d p.c. While mononuclear epithelial cells express the antigen only on lateral membranes, in oligonuclear cells the signal appears to be shifted to the basal cell membrane (arrow). Remnants of this basal location seem to persist in larger symplasms. Large symplasms exhibit, in addition, weak perinuclear cytoplasmic staining. l = Lumen. ×290.

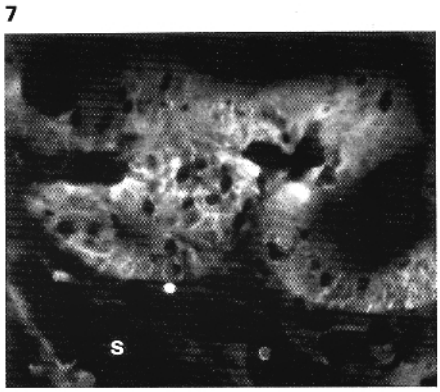


Fig. 7. CD44 immunofluorescence is reduced in the obplacental region of the implantation chamber on day 8 p.c. resulting in weak to moderate staining of lateral membranes of non-fused cells and of symplasms and in diffuse reactivity in the cytoplasm of symplasmic masses. s = Stroma. ×190.

shifted even further and was now found at the basal plasma membrane domain, a phenomenon that was most strikingly seen at 9 d p.c. (fig. 5c).

Slight differences in CD44 immunofluorescence between the different mucosal folds were seen from day 8 p.c. on in the implantation chamber: staining was reduced to an intermediate degree in the nonfused cells and small symplasms on placental and obplacental but not on paraplacental folds. However, a clear-cut gradient in staining intensity from implantation sites into blastocyst-free segments could not be established on longitudinal sections. Differences between the major mucosal folds appeared to result from the differences in symplasmic transformation of the epithelium and did not depend on the adhesion or simply the presence of the trophoblast. Where trophoblast had attached and started invasion, staining had been abolished completely. Nonattached trophoblast cells (at any stage) were only weakly reactive with faint staining occurring occasionally

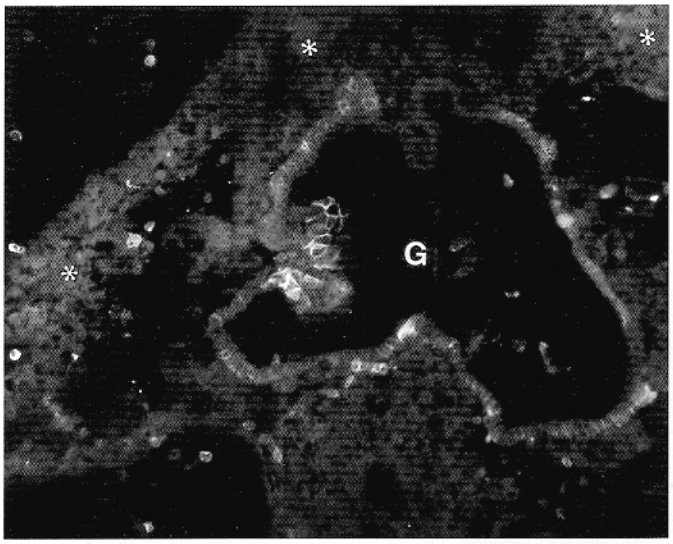


Fig. 8. The huge symplasmic masses (asterisks) at the placental fold in the implantation chamber at 10 d p.c. are free of specific CD44 immunofluorescence while, underneath, nonfused cells in remnants of the glands (G) display lateral staining in an inhomogeneous distribution. Strongly reacting individual cells, probably leukocytes, are seen in the endometrial stroma as well as in the uterine lumen. × 175.

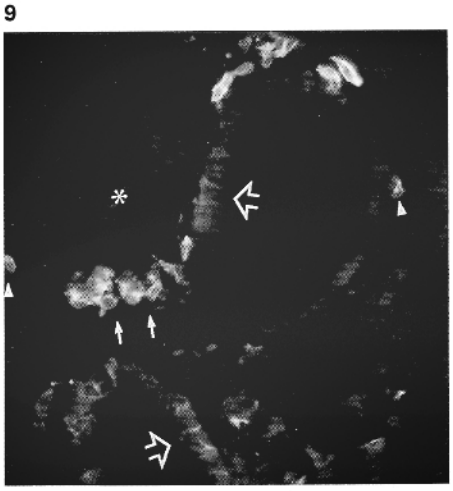
in the cytoplasm of cellular trophoblast as well as in trophoblastic knobs.

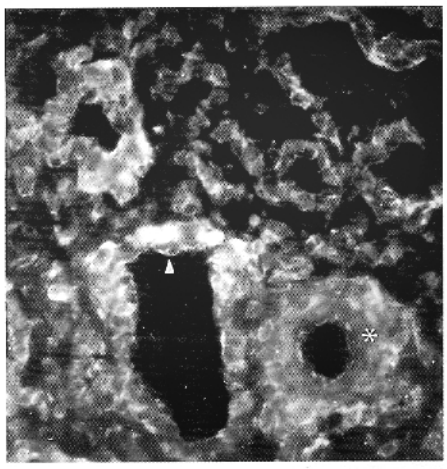
The expression of CD44 in *endometrial stroma* and *myometrium* during pregnancy was generally comparable to that observed in nonpregnant and pseudopregnant animals. One difference appeared to be an increase of the intensely reactive subpopulation of stromal cells in the implantation chamber after the onset of implantation. However, these cells were usually seen randomly distributed as individual cells or as small patches of aggregated cells in the stroma, the latter frequently underlying the large symplasmic masses in placental and obplacental areas (fig. 9). In addition, the perivascular decidual cells found in the implantation chamber from day 9 p.c. on exhibited fluorescence on their plasma membranes that was increased as compared to regular stromal fibroblasts (fig. 10). Staining of stromal elements, in pregnant as well as nonpregnant animals, was comparable to that described for human placenta with a prominent reaction on macrophage-like cells [St. Jacques et al., 1993].

In order to further confirm the differential expression of CD44 during pregnancy in rabbits, total cellular RNA of endometrium taken from interblastocyst segments on days 6 and 8 p.c. was analyzed by Northern blots (fig. 11). The signal detected in rabbit endometrium was in the range of about 1.6–1.7 kb and comparable in size and intensity to that obtained with rabbit lung tissue. The results paralleled those obtained at the protein level by immunofluorescence in that CD44 mRNA was detected on day 8 p.c. but not on day 6 p.c.

Discussion

In the experiments communicated here, the expression of the cell surface glycoprotein CD44 in rabbit endometrium during early pregnancy and pseudopregnancy was monitored by immunofluorescence and Northern blot analysis. CD44 comprises a family of multiple isoforms generated by alternative splicing and posttranslational modifications [Goldstein et al., 1989; Stamenkovic et al., 1989, 1991; Goldstein and Butcher, 1990; Brown et al., 1991; Günthert et al., 1991; review: Underhill, 1992]. One major isoform, CD44H, is found predominantly in cells of mesenchymal origin like fibroblasts and hematopoietic cells while another one, CD44E, is associated with subsets of epithelial cells [Flanagan et al., 1989; Brown et al., 1991; Stamenkovic et al., 1991; Mackay et al., 1994]. Whereas CD44H binds hyaluronic acid and other matrix molecules and is involved in cell motility and lymphocyte homing, CD44E does not display these activities and is thought to relate to cell-cell interactions [Aruffo et al., 1990; Sy et al., 1991; Thomas et al., 1992; Underhill, 1992]. Other isoforms appear to correlate with the metastatic potential of malignant cell types [Günthert et al., 1991]. Based on Northern blot analysis, it is difficult to identify the isoform of CD44 expressed in rabbit uterine epithelium (investigations on CD44 in the rabbit have not been reported so far). The rabbit molecule may not be completely homologous to the human molecule, from which the cDNA probe and the antibodies used here were derived. However, the RNA in rabbit uterine epithelium is similar in size to that in rabbit lung tissue. Since CD44E is usually expressed in the lung [Flanagan et al., 1989; Mackay et al., 1994] rabbit uterine CD44 may also be the epithelial form. On the other hand, the size of about 1.6–1.7 kb of the RNAs detected by Northern hybridization rather correlates with that of human CD44H [Stamenkovic et al., 1989, 1991; Brown, 1991], provided that human and rabbit CD44 RNA are similar in size. We cannot exclude the presence of other hyaluronic acid binding proteins in rabbit uterine epithelium like RHAMM [Hardwick et al., 1992] although the latter molecule could not be detected immunohistochemically in this tissue using different monoclonal antibodies (data not shown).





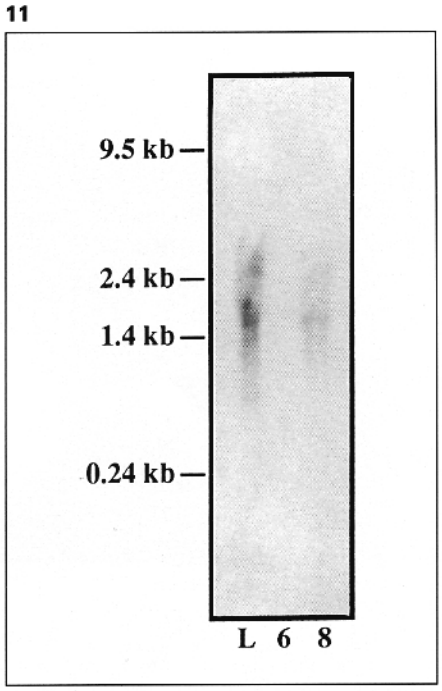


Fig. 9. Stromal cells and deeper crypts in pregnancy, day 8. A strongly CD44-positive subpopulation of stromal cells is found at random distribution (arrowheads) locally forming aggregates (arrows). These are frequently located in proximity of the large symplasmic masses (asterisk) on the placental and obplacental folds. While large symplasmic masses unveil only a perinuclear haze of CD44 fluorescence the mononuclear epithelial cells in the remains of the glands are labelled on the lateral membranes or are negative. ×290.

Fig. 10. Perivascular decidua. Detection of CD44 in the placental mucosal fold of the implantation chamber, 9 d p.c. Cells of the perivascular decidua show increased reactivity as compared to regular fibroblasts and smooth muscle cells in arterial walls (asterisk). Endothelial cells now also seem to have acquired some signal (arrowhead, c.f. fig. 1). ×290.

Fig. 11. Northern blot analysis of CD44 expression in pregnant rabbit endometrium. A positive signal is seen in an RNA band at 8 d p.c. (8) but not 6 d p.c. (6) and corresponds to a band in RNA extracted from rabbit lung tissue (L). Equal loading of the lanes was confirmed by fluorescence of the bands of 18 S and 28 S ribosomal RNAs after ethidium bromide staining of the gels (not shown).

The time schedule of the overall changes of reactivity patterns in the endometrium may suggest an association of this molecule with implantation. However, the absence of CD44 from the apical cell surface of uterine epithelium as well as of the trophoblast seems to rule out any direct role in trophoblast adhesion. On the other hand, the molecule could support penetration of the trophoblast through the uterine epithelium by changes in cell-cell adhesion. This appears possible from observations in stratified epithelia where CD44 is expressed in the basal and in the spinous layer but not in the upper layers [Flanagan et al., 1989; Brown et al., 1991; Mackay et al., 1994]. Although, based on its occurrence in the more basal layers of epithelia, the molecule is discussed to be associated with proliferating cells, it may well be that in stratified epithelia the expression of CD44 is rather related to the migratory potential enabling the cells to leave the basal layers. Therefore, the expression of CD44 in rabbit uterine epithelium may be connected with the considerable remodelling that this tissue undergoes in the periimplantation phase, finally enabling trophoblast penetration and invasion. Such functions would find further support if the rabbit uterine epithelial CD44 turns out to be CD44H since this isoform binds hyaluronic acid. In this case, interposition of hyaluronic acid could help reduce lateral cell-cell adhesiveness between epithelial cells which may also be diminished by partial redistribution of E-cadherin from the typical subapical location towards the basal pole [Donner et al., 1992; Denker, 1993]. This molecule, like CD44, accumulates at the basal domain of the cell membrane of small symplasms at the placental fold after 8 d p.c. [Donner et al., 1992; Denker, 1993]. Most probably, CD44 is located here, like E-cadherin, on the membranes of the small cytoplasmic projections of the epithelial cells that penetrate the basement membrane. This is an interesting phenomenon in the context of changes that take place in the polar organization of uterine epithelial cells during this phase.

In rabbit uterine epithelium CD44 is not correlated with epithelial proliferation which peaks on days 2 and 3 p.c. and which has more or less stopped at the time of implantation [Murai et al., 1981] when CD44 appears on the uterine epithelium. In addition, the lack of expression in the bottom parts of the glands contradicts an association with actively dividing cells as suggested by Brown et al. [1991] and Mackay et al. [1994] because in rabbit uterine epithelium the proliferating stem cells are mainly located in the deepest parts of the crypts [Conti et al., 1984]. From the observations on stratified epithelia it may alternatively be concluded that CD44 is affiliated with lower levels of differentiation. Whether this can be interpreted in such a way that uterine epithelial cells may revert, during acquisition of the receptive state, to a lower degree of differentiation possibly losing some epithelial properties will have to be tested. Changes in expression of integrin matrix receptors in human uterine epithelium were observed [Lessey et al., 1992] which may also reflect changes in cell-cell or cell-substrate adhesion. All these changes may be interpreted as representing a certain, perhaps only partial, conversion of polarity or partial loss of epithelial properties in general in periimplantative uterine epithelium as discussed previously [Denker, 1993] with respect to concepts on epithelio-mesenchymal transition. It would be of interest whether CD44 in rabbit uterine epithelium might represent the fibroblast-specific form, CD44H, which will have to be tested in subsequent investigations.

The establishment of the receptive state in the uterine epithelium is regulated by ovarian steroid hormones [Psychoyos and Casimiri, 1980]. However, CD44 expression in this system does not seem to be controlled by maternal steroid hormones alone as indicated by the very limited detection in pseudopregnant uteri (in which blastocysts are missing). Therefore, only the presence of blastocysts can elicit the pattern of CD44 expression described for the pregnant state implying the significance of embryonic signals. This is supported further by the observation that CD44 is expressed first in blastocyst-containing segments on day 6 p.c. before it appears in intersegments on day 7 p.c. Regulation by embryonic signals has been inferred previously for the upregulation of gap junctions in rabbit uterine epithelium with a similar time pattern as CD44 expression [Winterhager et al., 1988] and for the downregulation of aminopeptidase M (arylamidase) [van Hoorn and Denker, 1975]. However, differences appear to exist with respect to local effects: While connexin expression was clearly higher in the vicinity of blastocysts [Winterhager et al., 1988], the increase in CD44 immunofluorescence appeared to be only slightly accelerated here but was rather homogeneous over the length of the uterus (comprising implantation sites as well as intersegments) suggesting that for regulation of gap junction formation and of CD44 expression the types of signals or their mode of spreading may be different. Alternatively, hCG-induced pseudopregnancy may not mirror the corpus luteum-dependent transformation of rabbit endometrium as perfectly as previously thought. On the other hand, reduction of CD44 expression in the placental fold in the implantation chamber might well result from its inverse association with cell fusion. It will be interesting to see if unilateral tubal ligation experiments could support the observations on pseudopregnancy confirming the relevance of blastocyst-derived signalling. In the human, the control of CD44 expression in uterine epithelium may be dominated by maternal steroids. There, CD44 was expressed differentially during the menstrual cycle in uterine epithelium with strong upregulation at the beginning of the secretory phase [Albers et al., in press]. In contrast to our observations, Behzad et al. [1994] report no major variations of CD44 immunoreactivity in the human uterus.

In conclusion, differential expression of CD44 in rabbit uterine epithelium at the time of embryo implantation appears to be under the control of embryonic signals, acting on the basis of triggering by maternal steroid hormones. The exact pathway of signalling will have to be investigated. The molecule may be related to a certain state of differentiation required for trophoblast adhesion and invasion, but may well be involved in the process of trophoblast invasion after attachment has been established. Further studies on differential expression of the various CD44 isoforms in rabbit endometrium and functional assays may give valuable functional clues for this molecule in endometrial receptivity as well as insight into the control of expression of this molecule.

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