BIOLOGY OF REPRODUCTION **55**, 548–558 (1996)

Differential Expression of Two β-Galactoside-Binding Lectins in the Reproductive Tracts of Pregnant Mice[1]

Brooke Phillips, Keith Knisley, Kayla D. Weitlauf, Janelle Dorsett, Vaughan Lee, and Harry Weitlauf[2]

Departments of Cell Biology and Biochemistry and Ob/Gyn, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

ABSTRACT

Two β-galactoside-binding proteins were isolated from utero-placental complexes of pregnant mice and identified as the S-Lac lectins galectin-1 and galectin-3. The spatiotemporal pattern of appearance of those proteins was determined by immunocytochemistry. Galectin-1 was present in all tissue compartments of the uterus except the luminal and glandular epithelium. It was found in the uteri of animals from all preimplantation stages of pregnancy, as well as in those from nonpregnant, ovariectomized, or sexually immature animals. After implantation of the embryo, cells of the decidua basalis were labeled, as were granular metrial gland cells, all trophoblastic elements of the placenta, the myometrium, and nondecidualized endometrium. By contrast, there was little evidence of galectin-3 in the uteri of nonpregnant animals or during the preimplantation stages of pregnancy. However, immunoreactive material was observed in endometrial cells of the primary decidual zone immediately after implantation and at later stages of pregnancy in the decidua basalis, metrial gland, and all trophoblastic elements of the placenta. There was no evidence of galectin-3 in the myometrium or nondecidualized endometrium. After parturition, amounts of galectin-3 in the endometrium and metrial triangle appeared to decrease as the implantation sites were resorbed. These data suggested that the function of galectin-1 is one of tissue maintenance, whereas the function of galectin-3 is related specifically to pregnancy.

INTRODUCTION

It has been reported that large amounts of β-galactoside-binding proteins of the S-Lac lectin family are present in the rat uterus [1] and human placenta [2]. The S-Lac lectins (recently renamed galectins [3]) are characterized by a highly conserved carbohydrate recognition domain with a binding preference for *N*-acetyllactosamine and particularly the repeating structure poly-*N*-acetyllactosamine [4, 5]. Linkage of these proteins to a variety of important processes, including neoplastic transformation and tumor invasiveness, cellular proliferation, cell-cell, cell-matrix, and matrix-matrix interactions, and local immunomodulation, has generally been attributed to their ability to recognize specific glycoconjugates by means of that binding site (see [6–8] for reviews). Because of the presumed need for regulation of such processes during embryo implantation and placentation, the presence of large amounts of the galectins in the uterus and placenta is particularly provocative. We were prompted to undertake the present experiments by this and by the recent observations that the 14-kDa galectin-1 is expressed in trophoblastic cells of the mouse as early as the blastocyst stage [9] and that a protein having immunoreactivity with an antibody against the 30-kDa galectin-3 is present in mouse blastocysts and midpregnancy implantation sites [10, 11]. In this work, we have isolated and rigorously identified β-galactoside-binding proteins from the murine uteroplacental complex and determined the spatiotemporal patterns of their appearance during pregnancy.

MATERIALS AND METHODS

Animals and Breeding

Outbred Swiss mice were used for all studies (Hilltop Laboratories, Scottdale, PA). Virgin females (6–8 wk of age) were selected at random stages of the estrous cycle and placed with fertile males. Mating was confirmed by detection of a vaginal plug (Day 1 of pregnancy).

Isolation of Lactose-Binding Lectins

Animals were killed by cervical dislocation on Day 15 of pregnancy, and the uterine horns were opened along the antimesometrial side. Each embryo was removed from its placenta, and the uterine and placental tissues were rinsed three times in ice-cold PBS, weighed, and homogenized in Lac-MEPBS (300 ml/150 g tissue) at low speed with a Brinkmann tissue homogenizer (Brinkmann Instruments Co., Westbury, NY). Lac-MEPBS contains PBS (150 mM NaCl, 10 mM Na_2HPO_4, pH 7.2), β-mercaptoethanol (4 mM), EDTA (2 mM), and lactose (150 mM). The homogenate was centrifuged at 1200 × *g* for 20 min to remove cells and debris, and the supernatant was frozen (−20°C), thawed, and recentrifuged. The clarified supernatant was sterile filtered through a 0.2-μm membrane, dialyzed three times against 10 volumes of MEPBS (without lactose), and added slowly to a column (3.5 × 20 cm) containing lactosyl-Sepharose (150 ml packed gel; Sigma, St. Louis, MO). The column was washed extensively with MEPBS containing 100 mM sucrose until no further protein could be detected in the effluent using a Bradford kit (Bio-Rad, Richmond, CA). Lactose-binding lectins were then eluted with MEPBS containing 100 mM lactose and dialyzed three times against 10 volumes of 10 mM Tris, pH 8.2.

Antibodies

Polyclonal (rabbit) antiserum against the M_r 14 500 lectin from rat lung (anti-L-14 [i.e., anti-galectin-1]) was a gift from Dr. Hakon Leffler (Langley Porter Psychiatric Institute, University of California, San Francisco). Hybridomas secreting the rat IgG2a monoclonal antibodies M3/38 (anti-galectin-3, [12]) and 53–6.72 (anti-lyt2, [13]) were obtained from American Type Culture Collection (Rockville, MD). Cells were grown in Dulbecco's Modified Eagle's Medium containing glucose (4.5 mg/ml), penicillin (100 U/ml), streptomycin (10 mg/ml), and fetal bovine serum (5%). Culture supernatants were used as a source of monoclonal

Accepted April 23, 1996.
Received October 11, 1995.
[1]Support for this research was provided in part by a grant from the Howard Hughes Medical Institute through the Undergraduate Biological Sciences Education program and by funds from NIH (HD 29801) and the TTUHSC Laboratory for the Study of Reproduction.
[2]Correspondence. FAX: (806) 743–2747; e-mail: cbbhmw@ttuhsc.edu

antibody. Exhausted medium was centrifuged to remove cell debris, filtered through 0.2-μm membranes, and stored at −20°C. Antibody concentrations of the supernatants ranged from 20 to 50 μg/ml as determined by radial immunodiffusion. Polyclonal rabbit anti-mouse laminin was obtained from E-Y Laboratories (San Mateo, CA). Polyclonal (rabbit) anti-mouse fibronectin was obtained from Gibco-Bethesda Research Laboratories (Grand Island, NY). Pooled normal rabbit serum (NRS) was used as a negative control for all polyclonal rabbit antisera.

Specificity of Anti-Galectin-1 and Anti-Galectin-3

To test antibody specificity, uterine and placental tissues were removed from virgin females, and animals were killed on Day 1, 4, 8, 12, or 16 of pregnancy and 24 h postpartum (as described above). The tissue samples were homogenized in solubilization buffer (2% SDS, 0.0625 M Tris base, 10% glycerol, 2.5% β-mercaptoethanol), and comparable amounts of total protein were separated by SDS-PAGE (10%). Proteins were transferred to Immobilon-P paper (Millipore, Bedford, MA) using a Semiphor semi-dry blotting apparatus (Hoefer, San Francisco, CA), and the blots were placed in 2% BSA in PBS with 0.1% azide for at least 30 min. The blots were immersed in either anti-galectin-1 (1:2000 in PBS-0.5% Tween 20 [PBS-Tween]) or monoclonal anti-galectin-3 (1:500 in PBS-Tween) and were incubated for 1 h. NRS (1:2000 in PBS-Tween) was used as a negative control for anti-galectin-1, and the monoclonal 53–6.72 (1:500 in PBS-Tween) was used as an isotypic negative control for anti-galectin-3. The blots were then rinsed extensively in PBS-Tween and were placed for 1 h in one of two horseradish peroxidase-conjugated secondary antibodies. For anti-galectin-1 and its NRS control, the affinity-purified goat anti-rabbit IgG (Cappel, Durham, NC), preincubated 1:250 in normal mouse serum for 30 min, was used at a final concentration of 1:40 000 in PBS-Tween. For anti-galectin-3 and its control, the goat anti-rat IgG (with no cross-reactivity to mouse; Cappel) was used at a final concentration of 1:30 000 in PBS-Tween. After being rinsed in PBS (without Tween), the blots were immersed in PBS containing diaminobenzidine (DAB, Sigma; 0.6 mg/ml) and hydrogen peroxide (0.012%) until color developed.

Immunoreactivity of Lactose-Binding Proteins

Proteins that were specifically eluted from lactosyl-Sepharose with lactose were separated by SDS-PAGE (10%) and transferred to nitrocellulose. After transfer, the blots were rinsed several times with PBS, then rocked overnight at 4°C in PBS containing 2% BSA and 0.1% sodium azide. For detection of galectin-1, blots were placed in anti-galectin-1 (1:2000 in PBS-Tween), rocked for 1 h, washed extensively with PBS-Tween, and placed in horseradish peroxidase-conjugated anti-rabbit IgG (Cappel, 1:5000 in PBS-Tween) for 1 h. The blots were rinsed several times with PBS (without Tween) and placed in PBS containing DAB and hydrogen peroxide until color developed. For detection of galectin-3, blots were placed in 150 mM sodium acetate (pH 3.0) for 15 min, rinsed four times with PBS-Tween, and placed in PBS-Tween with 0.5% BSA for 15 min. Blots were then placed in monoclonal anti-galectin-3 supernatant containing 0.05% Tween, rocked for 1 h, washed extensively with PBS-Tween, and placed in alkaline phosphatase-conjugated goat anti-rat IgG (no cross-reactivity to mouse, Cappel; 1:1000 in PBS-Tween with 2% BSA) for 1 h. They were rinsed as before and then rinsed in staining buffer (100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5), and placed in 10 ml of the same buffer containing nitro blue tetrazolium (75 mg/ml; Sigma) and 5-bromo-4-chloro-3-indolyl phosphate (50 mg/ml, Sigma) until color developed.

Protein Sequencing

Lactose-binding lectins were concentrated by positive pressure dialysis, separated by SDS-PAGE on a 3-mm-thick gel, and electrophoretically transferred to nitrocellulose. The blot was stained briefly with Ponceau S (0.1% in 5% acetic acid, Sigma) and rinsed with 1% acetic acid to locate the protein bands. The bands were cut from the blot, rinsed with HPLC-grade water, and stored wet at −70°C in a sealed microfuge tube until tryptic digestion and sequencing could be performed by the Harvard Microchem facility (Cambridge, MA). The protein was digested with trypsin in situ. Tryptic peptides were eluted from the nitrocellulose and separated by reverse-phase HPLC. Optimal HPLC fractions were selected for sequencing by automated Edman degradation.

Immunocytochemistry

The uterine horns were removed from animals on the appropriate day of pregnancy and cut into cross sections containing an embryo, its placenta, and the adjacent uterine structures. The segments were fixed with 4% paraformaldehyde in sodium phosphate buffer (pH 7.3) for 24 h. The tissue was then dehydrated, embedded in Paraplast Plus (Sherwood Medical Labs, St. Louis, MO), and serially sectioned at 5 μm. The slides were deparaffinized and blocked with 1% BSA and normal goat serum to prevent nonspecific binding. Adjacent sections were incubated overnight with antibodies diluted in PBS with 1% BSA and 0.1% sodium azide: anti-galectin-3 (or its control 53–6.72 at 1:250), anti-galectin-1 (or its control NRS at 1:500), anti-laminin or anti-fibronectin (or their control NRS at 1:250). After exposure to antibodies, the slides were rinsed in PBS and placed in a horseradish peroxidase conjugate for 90 min. Slides probed with anti-galectin-3 and 53–6.72 required treatment with 1:400 goat IgG antibody to rat IgG (no cross-reactivity to mouse, Cappel). Slides probed with anti-galectin-1, anti-laminin, anti-fibronectin, and NRS required 1:3000 goat IgG antibody to rabbit IgG(Fc) (Cappel). Slides were then rinsed in PBS and placed in PBS containing DAB and hydrogen peroxide for 10 min. They were counterstained with Mayer's hematoxylin for 3 min, rinsed in H_2O, dipped in ammonium hydroxide (0.4%) bluing solution for 10 sec, dehydrated, and coverslipped with Permount (Fisher, Dallas, TX). In sections probed for laminin and fibronectin (and their controls), it was necessary to pretreat the slides with trypsin (1 mg/ml, Sigma) overnight before exposure to primary antibody; the remainder of the process was as described above. In some cases, with anti-galectin-1 and anti-galectin-3, a brief trypsinization (less than 1 h) was used to reduce cytoplasmic staining.

In several animals, a ligature was placed on one uterotubal junction on Day 1 of pregnancy to create a sterile pseudopregnant horn, and 10 μl of sesame oil was injected into the lumen at 1400 h on Day 4. Animals were killed on Days 7–11, and the resulting deciduomata were processed as described above. In a few cases, virgin animals were selected at random stages of the estrous cycle and ovariectomized bilaterally, allowed 1 wk to recover, and

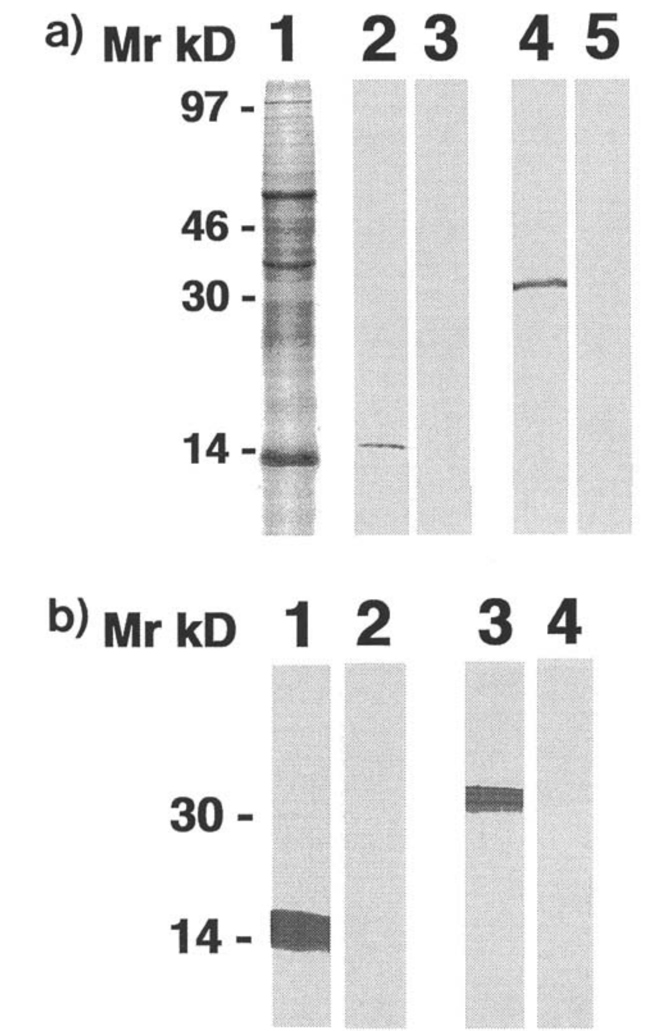


FIG. 1. Western analysis of uteroplacental proteins. **a**) Solubilized proteins from the mouse uteroplacental complex on 10% polyacrylamide gel (numbers on far left indicate molecular weight standards [× 10^3]). Lane 1 stained with Coomassie blue; lane 2 probed with anti-galectin-1, lane 3 with NRS, lane 4 with monoclonal anti-galectin-3, lane 5 with monoclonal isotypic antibody control 53–6.72. Anti-galectin-1 reacts with a 14-kDa protein, and anti-galectin-3 reacts with a 30-kDa protein. No cross-reactivity was observed between these antibodies and other proteins in the mouse uteroplacental complex. **b**) Lactose-binding proteins from the mouse uteroplacental complex. Lane 1 probed with anti-galectin-1, lane 2 with NRS, lane 3 with the monoclonal anti-galectin-3, lane 4 with the monoclonal isotypic antibody control 53–6.72. The anti-galectin-1 reacts with the 14-kDa protein, and anti-galectin-3 reacts with 30-kDa protein species; no cross-reactivity was observed.

given a regimen of estrogen and progesterone sufficient to induce maximum uterine sensitivity [14]. Segments of horns from these animals were also processed for immunocytochemistry as described above.

RESULTS

Preliminary experiments were undertaken with solubilized proteins from uterine and placental tissues taken at key days in pregnancy to determine specificity of our antibodies. The proteins were separated by SDS-PAGE, transblotted onto Immobilon-P paper, and probed with anti-galectin-1 or anti-galectin-3. The results of samples taken at Day 16 of pregnancy (Fig. 1a) show that with anti-galectin-1, a single band at 14 kDa was labeled, and with anti-galectin-3, a single band at 30 kDa was labeled. Similar results were obtained with samples taken from virgin females and from animals killed on Days 1, 4, 8, and 12 of pregnancy or at 24 h postpartum (data not shown). Additional preliminary experiments were undertaken to characterize soluble lactose-binding proteins from homogenates of mid-pregnancy implantation sites and placentas. Clarified supernatants were passed over a lactosyl-Sepharose column, and bound proteins that eluted with 100 mM lactose were separated by SDS-PAGE. Two major Coomassie blue bands were observed: one at 14 kDa and the other at 30 kDa (data not shown). Anti-galectin-1 reacted with transblots of the 14-kDa protein, and there was no cross-reactivity with the 30-kDa protein; anti-galectin-3 reacted with transblots of 30-kDa protein species, and there was no cross-reactivity with the 14-kDa protein (Fig. 1b). Amino

a) Galectin-1

1
RGEVASDAKSFVLNLGKDSNNLCLHFNPRFNAHGDANTIVCNTK**EDGTWGTEHR**EPAF

78 134
PFQPGSITEVCITFDQADLTIKLPDGHEFKFPNRL.**NLEAINYMAADGDFK**IKCVAFE

b) Galectin-3

1
MADSFSLNDALAGSGNPNPQGYPGAWGNQPAGAGGYPGAAY I PGAYPGQAP I PGAYPGQAP I PGAYPGQAP I

67
PSAYPGPTA I PGAYPGP.TA I PGAYPGQPA. I PGAFPGQPGA I PGAYPSA I PGGYPAAGP I YGVPAGPLTVPY

119
DLPLPGGVMPRMLITIMGTVKPNANRIVLDF.RR**GNDVAFHFNPR**FNEN.NRRVIVCNTKQDNNWGKEER**QSAF**

192 250
PFESGKPFKI**QVLVEADHF**K**VAVNDAHLLQYNHR**MKNLREISQLGISGDITLTSANHAMI

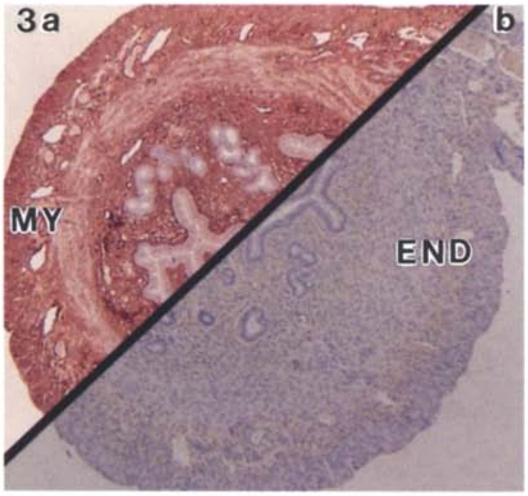
FIG. 2. Amino acid sequences of murine galectins. **a**) Amino acid sequences of murine galectin-1 (GenBank accession number X51903). Sequences of tryptic fragments of the 14-kDa protein from the uteroplacental complex were found to correspond with those of homologous segments of authentic galectin-1 (bold and underlined). **b**) Amino acid sequences of murine galectin-3 (GenBank accession number P16110). Sequences of several tryptic fragments of the 30-kDa protein were found to correspond with those of homologous segments of authentic galectin-3 (bold and underlined).

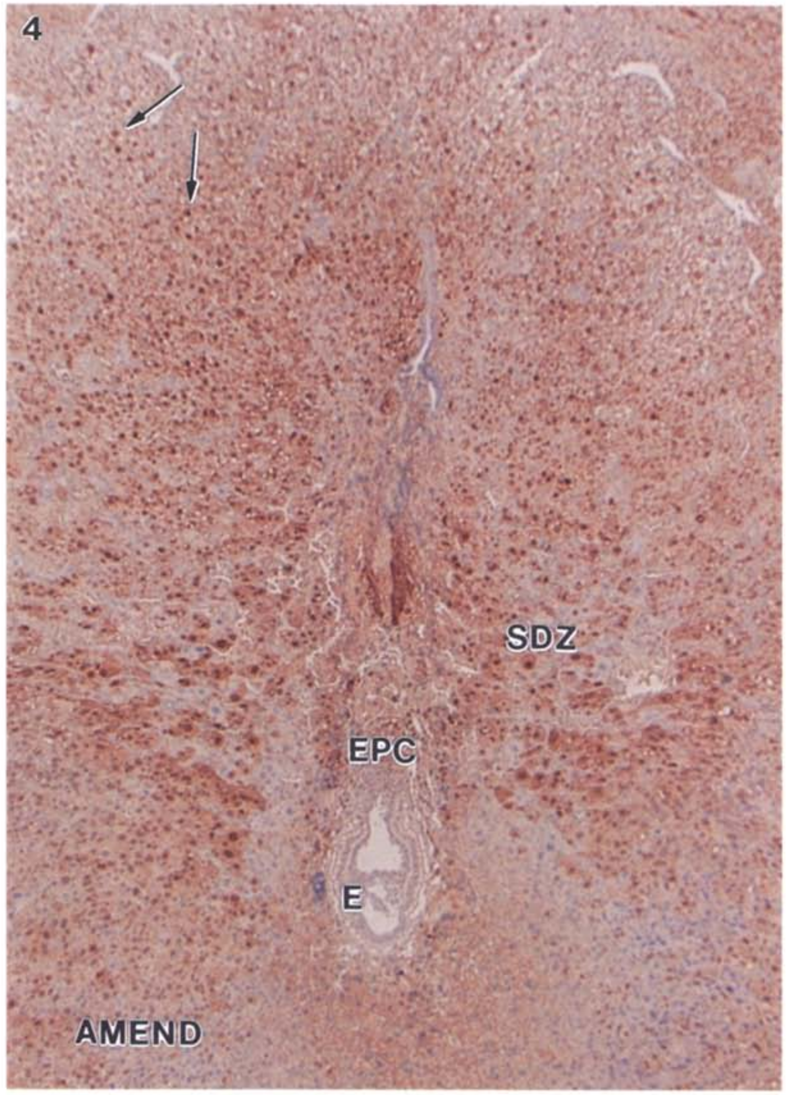
FIG. 3. **a**) Cross section of a mouse uterus from a virgin female stained with anti-galectin-1. ×32. Positive staining for galectin-1 is observed in all tissue compartments except the luminal and glandular epithelium. **b**) No reactivity was observed when NRS was substituted for the primary antibody. ×32. Adjacent section is shown in Figure 10 (anti-galectin-3). Abbreviations for Figures 3–16: AMEND, antimesometrial region of endometrium; DB, decidua basalis; EPC, ectoplacental cone; E, embryo; END, endometrium; FIS, former implantation site; GTC, giant trophoblast cell; GC, glycogen cells; MG, metrial gland; MY, myometrium; PL, placental labyrinth; PDZ, primary decidual zone; SDZ, secondary decidual zone; ST, spongy trophoblast; VS, vascular sinuses.

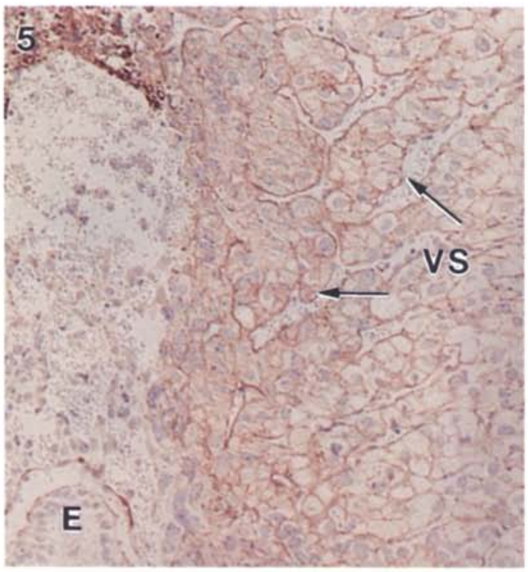
FIG. 4. Cross section through an implantation site at Day 8 of pregnancy stained with anti-galectin-1. ×60. Labeling appears to be both cytoplasmic and nuclear in the primary and secondary decidual zones. By contrast, cells are less heavily stained, and cell boundaries are more distinct in the antimesometrial regions of the endometrium. Numerous intensely labeled cells (arrows) are present in the mesometrial endometrium (also see Fig. 6). Cells of the ectoplacental cone are labeled, while those of the embryo proper are unreactive. Adjacent section is shown in Figure 11 (anti-galectin-3).

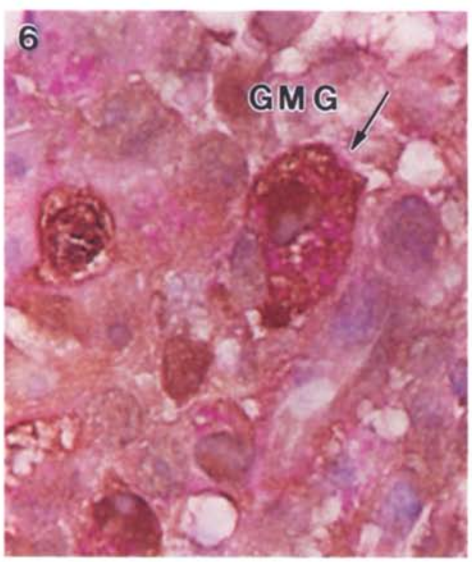
FIG. 5. Cross section through an implantation site at Day 8 of pregnancy stained with anti-galectin-1 after brief trypsinization. Positive immunostaining clearly outlines the vascular sinuses (arrows). ×129.

FIG. 6. Cross section through the mesometrial region of the endometrium on Day 8 stained with anti-galectin-1 and PAS. Numerous cells scattered throughout the area were intensely labeled with anti-galectin-1 and had PAS-positive granules (i.e., granular metrial gland cells). ×806.









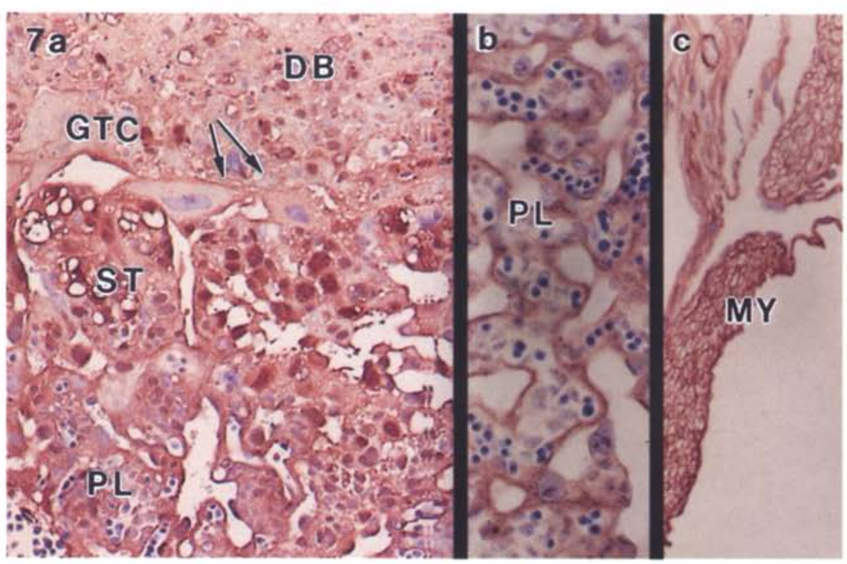


FIG. 7. **a**) Cross section of a Day 12 uteroplacental complex stained with anti-galectin-1. Nuclei of the cells of the decidua basalis are heavily stained, while immunoreactivity in the cytoplasm is variable. Cytoplasm of the giant trophoblast cells (arrows) is lightly labeled and cell outlines can be discerned, but no reactivity is observed in the nuclei of these cells. Both cytoplasm and nuclei of trophoblast cells in the spongy and labyrinth portions of the placenta show positive immunostaining. ×129. **b**) Cross section through the placenta at Day 12 of pregnancy stained with anti-galectin-1 after brief trypsinization. Positive staining is observed on the surface of the vascular spaces in the placental labyrinth. ×200. **c**) Intense staining is seen in pericellular connective tissue of the myometrial compartment. ×300.

acid sequences of several tryptic fragments of the 14-kDa protein were found to be identical to corresponding regions of murine galectin-1, and those of several tryptic fragments of the 30-kDa protein were found to be identical with corresponding regions of murine galectin-3 (GenBank accession numbers X51903 and P16110, respectively; Fig. 2). Similarities in size, immunoreactivity, and identity in amino acid sequences indicate that the 14-kDa and 30-kDa lactose-binding proteins from the uteroplacental complex were authentic murine galectin-1 and galectin-3, respectively. The multiple immunoreactive 30-kDa species shown in lane 3 of Figure 1b possibly represent splicing variants [15, 16] and/or posttranslational modifications [17, 18] of galectin-3. Further, it was found that the yield of both galectins increased several fold when lactose was included in the original homogenization buffer (data not shown), and it was presumed that these proteins were bound to glycoconjugates in situ.

Anti-galectin-1 immunoreactive material was found in all tissue compartments of the uterus and placenta except the luminal and glandular epithelium. Before implantation, label was distributed throughout the myometrium and endometrial stroma. The cytoplasm was consistently labeled, nuclei were variably labeled, and outlines of myometrial cells and vascular structures were clearly demarcated. A similar pattern was observed in nonpregnant uteri from all stages of the estrous cycle as well as from ovariectomized animals (i.e., with and without ovarian steroid hormone replacement) and sexually immature females (Fig. 3a). There was no obvious change in labeling at the time of implantation on Day 5 of pregnancy (not shown). By Day 8 of pregnancy, the nuclei as well as cytoplasm of cells undergoing decidualization (in both the primary and secondary decidual zones) were heavily stained. Cells of the ectoplacental cone were also labeled, but cells in the embryo proper were not. Although labeling in the anti-mesometrial region of the endometrium appeared to become less intense as the decidual mass increased in size, cell boundaries were quite distinct (Fig. 4). Short incubations of the sections in trypsin before exposure to anti-galectin-1 reduced cytoplasmic labeling to a finely reticular pattern and revealed strong localization to the surfaces of the vascular sinuses in the endometrium (Fig. 5). In addition, there were intensely labeled cells scattered throughout the mesometrial endometrium and the metrial gland. These cells were found to contain periodic acid-Schiff (PAS)-positive granules (Fig. 6) and were presumed to be the subpopulation of modified natural killer leukocytes referred to as granular metrial gland cells (GMG cells [19]). By Day 12 of pregnancy, the decidua basalis and definitive placenta were clearly established. The nuclei of cells in the decidua basalis were consistently stained, while staining of the cytoplasm was variable. Cytoplasm of the giant trophoblast cells was lightly labeled, and cell outlines could be discerned, but the majority of nuclei were negative. Trophoblast cells in the labyrinth region of the placenta were strongly reactive, as were most of those in the spongy layer (Fig. 7a). Staining after brief trypsinization revealed labeling of the surface of vascular spaces in the placental labyrinth (Fig. 7b), as well as of pericellular connective tissue in the myometrium (Fig. 7c). Although there was little change in the pattern of labeling at later stages of pregnancy, the so-called placental glycogen cells [20], which appeared around Day 14 and eventually replaced much of the decidua basalis, were also strongly positive (Fig. 8). After parturition, the distribution of anti-galectin-1 immunoreactive material was similar to that which occurred before pregnancy (Fig. 9).

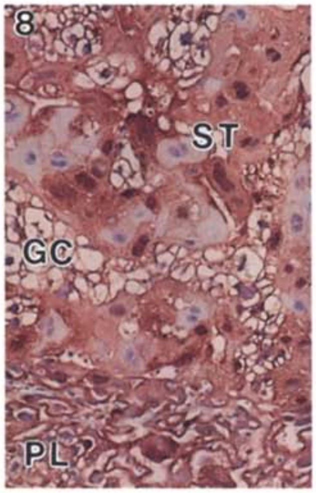
The uterine distribution of galectin-3 was strikingly different from that of galectin-1. Before implantation, anti-galectin-3 immunoreactive material was observed in only a few cells scattered throughout the endometrium, while the remainder of the uterus, including the endometrial stroma and the myometrium with its associated pericellular connective tissue, was not labeled (Fig. 10). Soon after implantation on Day 5 of pregnancy, a few endometrial cells immediately adjacent to the embryo were found to be reactive with anti-galectin-3 (not shown). Cells of the primary and secondary decidual zones were found to be strongly labeled by Day 8. Cells in the mesometrial regions were labeled in both the nucleus and cytoplasm but had indistinct cell boundaries. Those cells in the antimesometrial regions had conspicuously less intracellular labeling but cell boundaries were more pronounced (Fig. 11). The remainder of the endometrium, the myometrium and its associated connective tissue, the ectoplacental cone, and cells of the embryo proper were not labeled. Short incubations of the slides with trypsin before exposure to anti-galectin-3 to reduce cytoplasmic staining revealed that the basal lamina of the embryo was lightly labeled. However, the basal laminae of vascular structures, uterine epithelium, and the surfaces of the vascular sinuses were not labeled (Fig. 12). In addition, numerous GMG cells scattered throughout the endometrium between the embryo and the metrial gland were intensely labeled with anti-galectin-3 (Fig. 13). By Day 12, the cells of the decidua basalis were found to react strongly with anti-galectin-3, while the more peripheral nondecidualized endometrium and the myometrium remained unlabeled. Trophoblast cells in the labyrinth region were heavily stained, as were most cells of the spongy layer and metrial gland; giant trophoblast cells were weakly reactive (Fig. 14a). Short incubations of the sections with trypsin before exposure to anti-galectin-3 revealed no immunoreactive material on the surfaces of the placental labyrinth (Fig. 14b) or connective tissue of myometrium (Fig. 14c). At a slightly later stage of pregnancy, the placental glycogen cells appeared and were strongly immunoreactive (Fig. 15). Amounts of anti-galectin-3 reactive material in the endometrium and metrial gland appeared to decrease after parturition (Fig. 16). The decidualized endometrium of artificially induced deciduomata was also positively

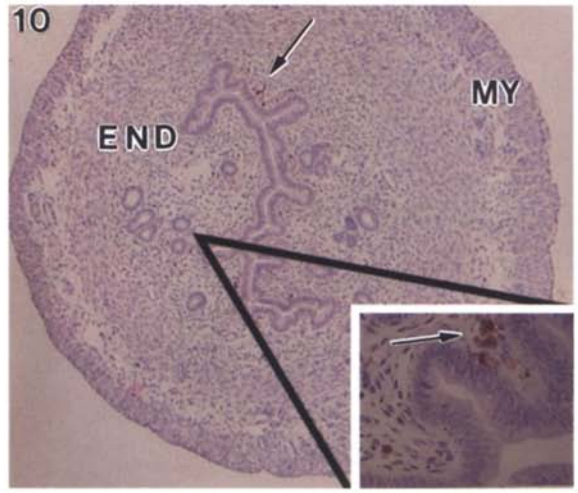
FIG. 8. Cross section of a Day 16 uteroplacental complex stained with anti-galectin-1. The decidua basalis is greatly reduced in amount and is largely replaced by placental glycogen cells. The spongy trophoblast is variably labeled, and all other elements of trophoblastic origin, including the glycogen cells, are strongly immunoreactive. ×107.

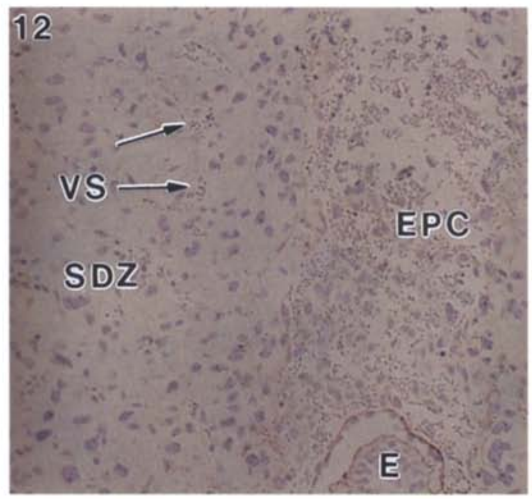
FIG. 9. Cross section of a mouse uterus through an implantation site 24 h after parturition stained with anti-galectin-1. ×21. Smooth muscle and pericellular connective tissue of the myometrium, the endometrium, and the metrial gland are labeled while luminal epithelium is unreactive. Adjacent section is shown in Figure 16 (anti-galectin-3).

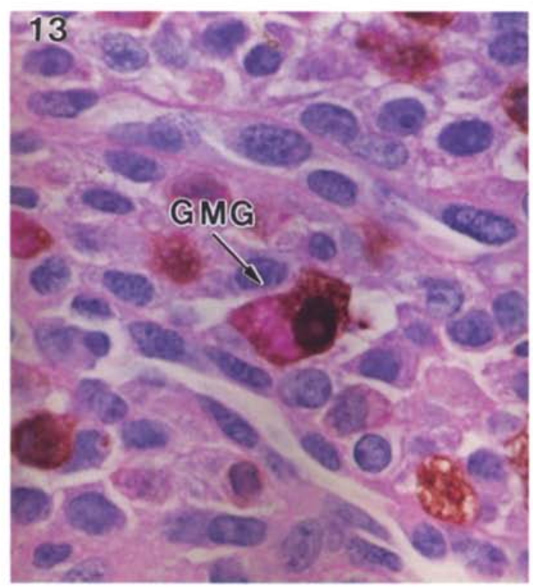
FIG. 10. Cross section of a mouse uterus from a virgin female stained with anti-galectin-3. ×40. The myometrial and endometrial compartments are not labeled although occasional immunoreactive cells (arrows) can be seen scattered throughout the endometrium (see insert ×160). Adjacent section is shown in Figure 3 (anti-galectin-1).

FIG. 11. Cross section through an implantation site at Day 8 of pregnancy stained with anti-galectin-3. ×60. Cells with strongly staining cytoplasm and nuclei are seen in the primary and secondary decidual zones dorsi-lateral to the embryo. In the antimesometrial region, staining is





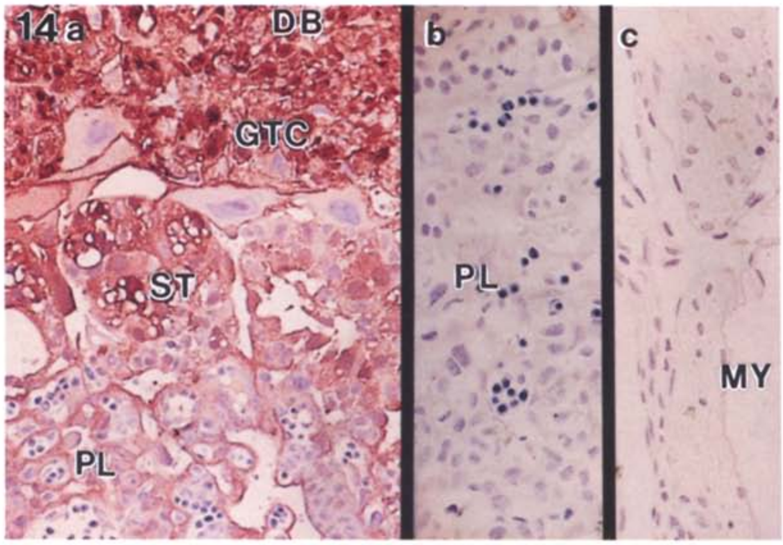


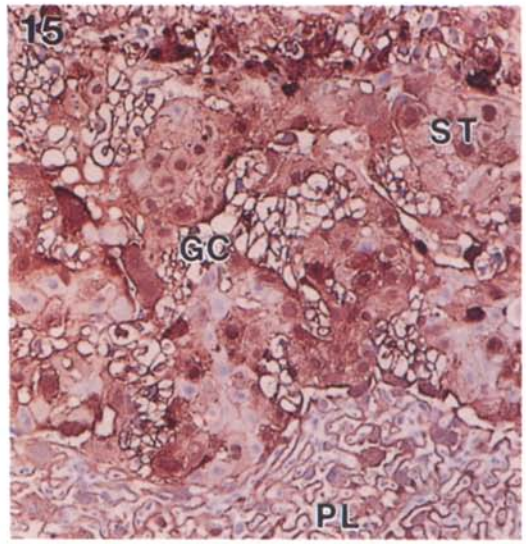


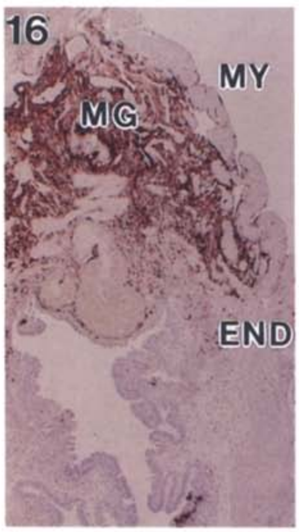
more prominent at the cell boundaries. Numerous intensely labeled cells (arrows) are present in the mesometrial regions of the endometrium (also see Fig. 13). No reactivity was observed in trophoblast cells in the ectoplacental cone or in the embryo proper. Adjacent section is shown in Figure 4 (anti-galectin-1).

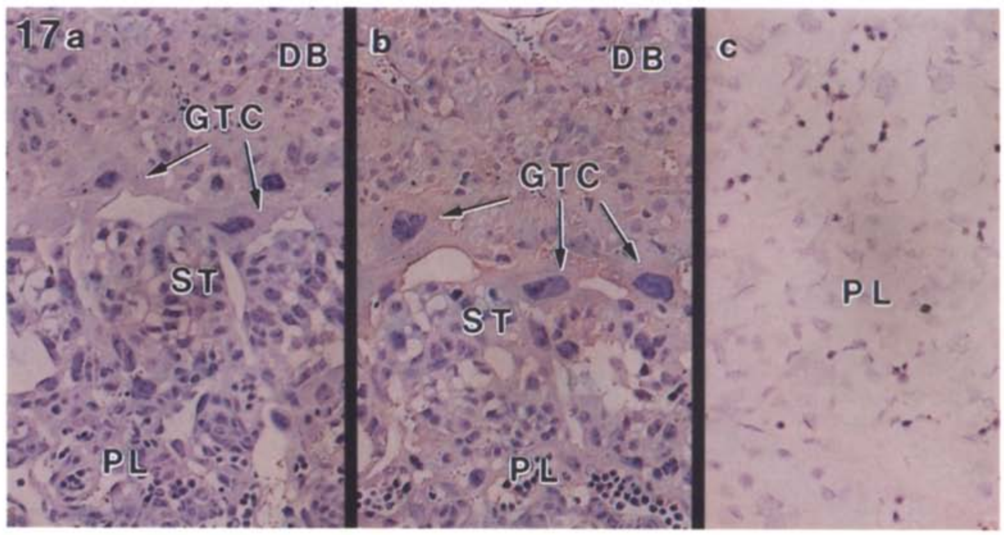
FIG. 12. Cross section through an implantation site at Day 8 of pregnancy stained with anti-galectin-3 after brief trypsinization. The basal lamina associated with the embryo is lightly stained; surfaces of the vascular sinuses are unreactive. ×129.

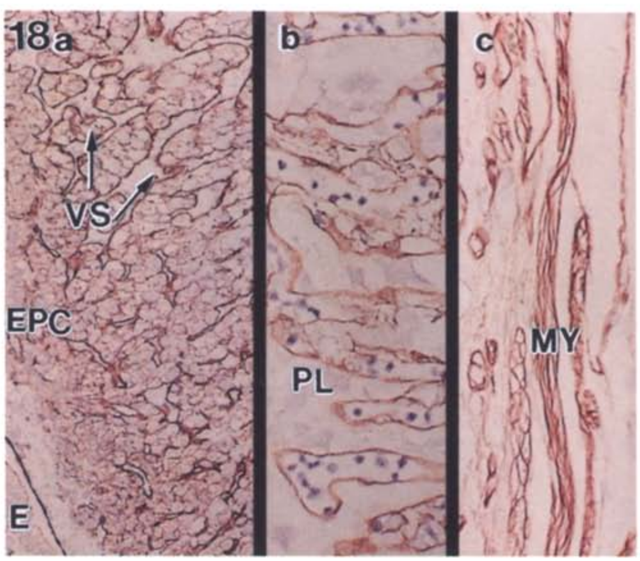
FIG. 13. Cross section through an implantation site at Day 8 of pregnancy stained with anti-galectin-3 and PAS. Many of the granular metrial gland cells (i.e., cells containing PAS-positive granules) are intensely labeled with anti-galectin-3. ×770.











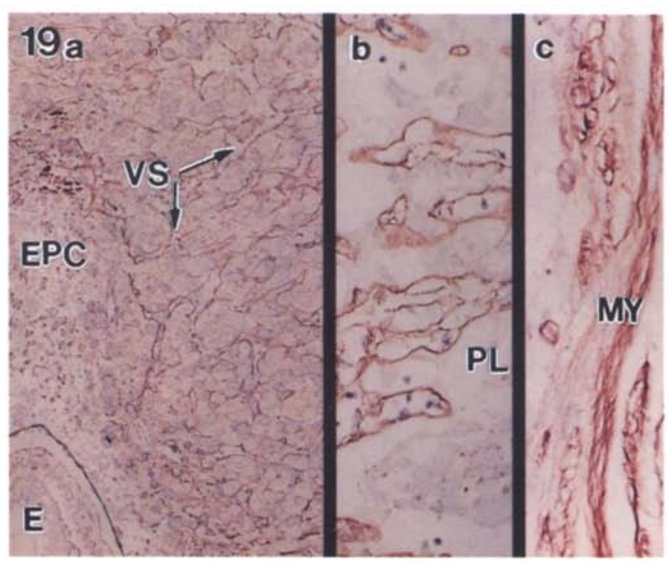


FIG. 14. Cross section of a Day 12 uteroplacental complex stained with anti-galectin-3. **a**) Heavy staining is observed in the cytoplasm and nuclei of cells of the decidua basalis. Trophoblast cells in the placental labyrinth are heavily stained, as are cells of the spongy layer; giant trophoblast cells appear to be only slightly reactive. ×129. **b**) Staining with anti-galectin-3 after brief trypsinization revealed little evidence of immunoreactivity on the surfaces of the vascular spaces of the placental labyrinth (×216) or **c**) the pericellular connective tissue of the myometrium (×300).

FIG. 15. Cross section of a Day 16 uteroplacental complex stained with anti-galectin-3. The glycogen cells, as well as most cells of all other trophoblast elements, appear to be labeled. ×107.

stained with anti-galectin-3 (not shown). Control sections are shown in Figure 17.

Labeling in sections stained with anti-laminin was strongly localized to the surfaces of the vascular sinuses in the endometrium (Fig. 18a). The basal laminae of blood vessels and of the embryo proper were labeled; trophoblast lining maternal vascular channels in the placental labyrinth was reactive (Fig. 18b) as was the pericellular connective tissue in the myometrium (Fig. 18c). This pattern of expression remained unchanged throughout the advancing pregnancy (not shown). Anti-fibronectin was localized to the same extracellular structures (Fig. 19, a-c). In addition, a finely reticular pattern of immunoreactive fibronectin was observed throughout the cytoplasm of cells in the endometrium and placenta, around decidual cells, while a more granular pattern appeared within the nuclei.

DISCUSSION

The present results demonstrate that the mouse uteroplacental complex contains two lactose-binding proteins with molecular masses of 14 kDa and 30 kDa. Their identities were established as murine galectin-1 and galectin-3, respectively, by demonstrating immunoreactivity with antibodies raised against the known β-galactoside-binding lectins L-14 (galectin-1) and Mac-2 (galectin-3). Furthermore, the amino acid sequences of several tryptic fragments had complete correspondence with those in homologous portions of the authentic proteins. These findings confirm and extend the observations that in several species β-galactoside-binding proteins are abundant in placentas and the female reproductive tract [1, 2, 9, 11, 21, 22].

Because of their ability to recognize specific complementary binding partners, the galectins have generally been perceived as elements in cognitive cell-cell or cell-matrix interactions or as intercellular regulators. Important biological processes to which they have been linked include neoplasia, tumor invasiveness and metastasis, regulation of cellular proliferation, connective tissue reorganization and differentiation, and local immunomodulation. Implantation and placentation presumably involve similar processes, and it was anticipated that, if the galectins do provide a molecular basis for some aspects of pregnancy, the spatiotemporal patterns of their appearance in the utero-placental complex might provide clues to their roles.

Evidence linking galectins to neoplasia and metastasis, and thus ostensibly to invasiveness of the placenta, is largely circumstantial. First, amounts of the galectins appear to increase with advancing malignancy ([23–25], but see [26]), as well as in oncogene transformed 3T3 cells [27]. Furthermore, overexpression of galectin-1 by mouse BALB3T3 cells resulted in a transformed phenotype [28], while transfection of weakly metastatic fibrosarcoma cells with galectin-3 cDNA resulted in transformation to a strongly metastatic phenotype [29]. It was anticipated that, if galectins are involved in the cancer-like invasion of the uterus by the placenta, there might be changes in the amounts or distribution of these proteins in the trophoblast during the course of pregnancy [22]. The findings that galectin-1 was ubiquitous in the murine placenta and uterus, and did not appear to change during the course of pregnancy, and that galectin-3 was also widely distributed on both sides of the fetomaternal interface did not fulfill that expectation.

That galectin-1 causes increased proliferation of vascular smooth muscle and pulmonary endothelial cells in vitro [30] and acts as a cytostatic factor for embryonic fibroblasts [31] establishes it as a growth factor. By contrast, evidence for such a function of galectin-3 is more circumstantial. First, galectin-3 is found predominantly as a phosphorylated protein in the nuclei and cytoplasm of quiescent cells, but as an unphosphorylated protein in the nucleus in proliferating cells [17, 18]. Thus, its synthesis, posttranslational modification, and intracellular location appear to be related to proliferative capacity [32–36]. Second, galectin-3 has limited homology in the N-terminal domain with proteins of the heterogeneous nuclear ribonucleoprotein (hnRNP) complex [37], is apparently a component of the hnRNP complex [38], and has homology with the transcription factor binding the c-*fos* serum response element [39]. Although the implication of these findings is that galectin-3 also is involved in regulating cellular proliferation, the cause and effect relationships remain unknown. In the present studies, galectin-1 and galectin-3 were both found in the nuclei of some cells in the uteroplacental complex and in the cytoplasm of others. However, because the histologic studies were performed on fixed material, it was not possible to correlate intracellular localization of the galectins to the proliferative status of a given cell.

The notion that galectins are involved in organization of certain elements of the extracellular matrix during tissue reorganization and differentiation is also based on anecdotal evidence. First, it is known that galectins have a preference for the poly-*N*-acetyllactosamine sequence, which accounts for much of the carbohydrate on laminin and tissue fibronectin [40–46]. Galectin-1 is a homodimer under physiologic conditions, and it has generally been supposed that it would bind and cross-link those matrix elements. Indeed, such binding can be demonstrated in vitro [47, 48]. Second, in immunohistochemical studies, galectin-1 was found distributed along with connective tissue elements that establish the basic architectures of various adult and embryonic tissues [21, 49, 50]. Third, tissue levels of the 14-kDa lectin have been reported to change with development and differentiation [6, 7, 49, 50–58]. It has been inferred from those observations that variations in amounts of galectin-1 either cause, or are caused by, reorganization of connective

FIG. 16. Cross section of a mouse uterus through an implantation site 24 h after parturition stained with anti-galectin-3. ×21. There is immunoreactive material in the metrial gland, and small amounts are present in the endometrium surrounding the former implantation site. Adjacent section is shown in Figure 9 (anti-galectin-1).

FIG. 17. Cross sections through the uteroplacental complex on Day 12 of pregnancy used for negative controls. No reactivity was observed with: **a**) 53-6.72 or **b**) NRS at 1:500, or **c**) when either anti-laminin or anti-fibronectin was replaced with NRS (1:250) after trypsinization of the tissue. ×200.

FIG. 18. Cross section through the uteroplacental complex stained with anti-laminin. **a**) Outlines of the vascular sinuses are clearly demarcated. (Day 8 of pregnancy; ×100). **b**) The basal lamina on the fetal surface of vascular channels in the placental labyrinth is sharply defined by immunoreactive staining. (Day 12; ×200). **c**) The pericellular connective tissue in the myometrial compartment is reactive (Day 12; ×300). Adjacent sections are shown in corresponding panels of Figure 19 (anti-fibronectin).

FIG. 19. Cross section through the uteroplacental complex stained with anti-fibronectin. **a**) Outlines of the vascular sinuses are clearly demarcated (Day 8; ×100). **b**) The basal laminae on the fetal surface of vascular channels in the placental labyrinth are labeled (Day 12; ×200). **c**) The pericellular connective tissue in the myometrial compartment is stained (Day 12; ×300). Adjacent sections are shown in corresponding panels of Figure 18 (anti-laminin).

tissue during differentiation. That, along with the recent observation that several putative regulatory elements exist upstream of the human and mouse genes [59, 60], supports the theory of its regulation by physiologic or developmental mechanisms. Hence, it was anticipated that in the present work correlations might be found between physiological events in pregnancy and changes in amounts or distribution of galectin-1 in the extracellular matrix. There was evidence of colocalization of galectin-1 with laminin and fibronectin in some areas of the nonpregnant uterus and the uteroplacental complex. However, there was little evidence of significant changes in its expression or distribution with sexual maturation and exposure to cyclic ovarian steroid hormones. No change in galectin-1 was seen with the establishment of pregnancy and the presumed connective tissue reorganization necessary to accommodate embryo implantation, growth of the conceptus, or parturition.

The carbohydrate recognition domain of galectin-3 is highly homologous to that of galectin-1, and, like that of the 14-kDa lectin, it shows a preference for the poly-*N*-acetyllactosamine sequence. Galectin-3 has been shown to bind mouse tumor laminin and tissue fibronectin in vitro [61–65], and it is capable of forming multimers [8, 66, 67]. Although galectin-3 has the potential to play a role in mediating or modulating cell-cell and cell-matrix interactions, there was not strong evidence of colocalization with laminin or fibronectin in the uteroplacental complex. On the other hand, galectin-3 is commonly found associated with the surfaces of various subpopulations of leukocytes, where it is presumed to be important for tissue infiltration and/or fixation of macrophages [12, 15, 62, 68–73], basophilic/mast cells [74, 75], and eosinophils [76]; furthermore, it has also been implicated as a local immunomodulatory factor in inflammatory reactions involving IgE [8]. Macrophages, basophils, and eosinophils are rare in the implantation sites of rodents [77–81], and it seems unlikely that the massive amount of galectin-3 in the primary and secondary decidual zones is present to support infiltration and fixation of those cells. However, the GMG cells are a prominent feature in the implantation sites of mice [82, 83]. Although the role of GMG cells is unknown, and the present results do not provide information about the functional relationship between galectin-3 and the GMG cells, the possibility that it is involved in localization or modulation of their immune functions is intriguing.

Results of the present experiments did not convincingly link the large amounts of galectins present in the uterus and placenta to any of the processes generally believed to be part of implantation or placentation. However, important new information about pregnancy was obtained in these studies. First, it is clear that in the uterus and placenta the expression of galectin-1 and the expression of galectin-3 are under differential control. Second, galectin-1 is expressed constitutively in essentially all tissue compartments of the nonpregnant as well as the pregnant uterus and placenta, whereas expression of galectin-3 is associated exclusively with pregnancy, and specifically with those tissues forming the fetomaternal interface. These latter findings indicate that, whereas galectin-3 appears to have a unique role in pregnancy, the function of galectin-1 in the uterus and placenta is not unique to pregnancy and seems more likely to be one of tissue maintenance.

ACKNOWLEDGMENTS

We thank Carol Cathey, Mary Hastert-Casadonte, and Angie Lee for their excellent technical assistance.

REFERENCES

1. Powell JT. Purification and properties of lung lectin. Biochem J 1980; 187:123–129.
2. Hirabayashi J, Kasai K. Human placenta β-galactoside-binding lectin. Purification and some properties. Biochem Biophys Res Commun 1984; 122:938–944.
3. Barondes SH, Castronovo V, Cooper DNW, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu FT, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirer F, Raz A, Rigby PWJ, Rini JM, Wang JL. Galectins: a family of β-galactoside-binding lectins. Cell 1993; 76:597–598.
4. Hirabayashi J, Kasai K. The family of metazoan metal-independent β-galactoside-binding lectins: structure, function and molecular evolution. Glycobiology 1993; 3:297–304.
5. Barondes SH, Cooper DNW, Gitt MA, Leffler H. Galectins. Structure and function of a large family of animal lectins. J Biol Chem 1994; 269:20807–20810.
6. Harrison FL. Soluble vertebrate lectins: ubiquitous but inscrutable proteins. J Cell Sci 1991; 100:9–14.
7. Harrison FL. Soluble β-galactoside-binding lectins in vertebrates. Lectin Rev 1991; 1:17–39.
8. Liu FT. S-type mammalian lectins in allergic inflammation. Immunol Today 1993; 14:486–490.
9. Poirer F, Timmons PM, Chan CTJ, Guénet JL, Rigby PWJ. Expression of the L14 lectin during mouse embryogenesis suggests multiple roles during pre- and post-implantation development. Development 1992; 115:143–155.
10. Weitlauf HM, Knisley KA. Changes in surface antigens on preimplantation mouse embryos. Biol Reprod 1992; 46:811–816.
11. Knisley KA, Weitlauf HM. Compartmentalized reactivity of M3/38 (anti-Mac-2) and M3/84 (anti-Mac 3) in the uterus of pregnant mice. J Reprod Fertil 1993; 97:521–527.
12. Ho MK, Springer TA. MAC-2, a novel 32 000 M_r mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. J Immunol 1982; 128:1221–1228.
13. Ledbetter JA, Herzenberg LA. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol Rev 1979; 47:63–90.
14. Finn CA, Martin L. Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. Biol Reprod 1972; 7:82–86.
15. Cherayil BJ, Weiner SJ, Pillai S. The mac-2 antigen is a galactose-specific lectin that binds IgE. J Exp Med 1989; 170:1959–1972.
16. Gritzmacher CA, Mehl VS, Liu FT. Genomic cloning of the gene for an IgE-binding lectin reveals unusual utilization of 5′ untranslated regions. Biochemistry 1992; 21:9533–9538.
17. Cowles EA, Agrwal N, Anderson RL, Wang JL. Carbohydrate-binding protein 35. J Biol Chem 1990; 265:17706–17712.
18. Huflejt ME, Turck CW, Lindstedt R, Barondes SH, Leffler H. L-29, a soluble lactose-binding lectin, is phosphorylated on serine 6 and serine 12 *in vivo* and by casein kinase I. J Biol Chem 1993; 268: 26712–26718.
19. Stewart IJ. Granulated metrial gland cells: pregnancy specific leukocytes? J Leukocyte Biol 1991; 50:198–207.
20. Redline RW, Lu CY. Localization of fetal major histocompatibility complex antigens and maternal leukocytes in murine placenta. Lab Invest 1989; 61:27–36.
21. Wasano K, Hirakawa Y, Yamamoto T. Immunohistochemical localization of 14 kDa β-galactoside-binding lectin in various organs of rat. Cell Tissue Res 1990; 259:43–49.
22. Van Den Brule FA, Price J, Sobel ME, Lambotte R, Castronovo V. Inverse expression of two laminin binding proteins 67LR and galectin-3, correlates with the invasive phenotype of trophoblastic tissue. Biochem Biophys Res Commun 1994; 201:388–393.
23. Raz A, Lotan R. Endogenous galactoside-binding lectins: a new class of functional tumor cell surface molecules related to metastasis. Cancer Metastasis Rev 1987; 6:433–452.
24. Raz A, Carmi P, Pazerini G. Expression of two different endogenous galactoside-binding lectins sharing sequence homology. Cancer Res 1988; 48:645–649.
25. Iramura T, Matsushita Y, Sutton RC, Carralero D, Ohannesian DW, Cleary KR, Ota DM, Nicolson GL, Lotan R. Increased content of an endogenous lactose-binding lectin in human colorectal carcinoma progressed to metastatic stages. Cancer Res 1991; 51:387–393.
26. Lotz MM, Andrews CW Jr, Korzelius CA, Lee EC, Steele GD Jr, Clarke A, Mercurio AM. Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are as-

sociated with the neoplastic progression of colon carcinoma. Proc Natl Acad Sci USA 1993; 90:3466–3470.

27. Raz A, Meromsky L, Zvibel I, Lotan R. Transformation related changes in the expression of endogenous cell lectins. Int J Cancer 1987; 39:353–360.
28. Yamaoka K, Ohno S, Kawasaki H, Suzuki K. Overexpression of a β-galactoside binding protein causes transformation of BALB3T3 fibroblast cells. Biochem Biophys Res Commun 1991; 179:272–279.
29. Raz A, Zhu D, Hogan V, Shah N, Raz T, Karkash R, Pazerini G, Carmi P. Evidence for the role of 34 kDa galactoside-binding lectin in transformation and metastasis. Int J Cancer 1990; 46:871–877.
30. Sanford GL, Harris-Hooker S. Stimulation of vascular cell proliferation by β-galactoside specific lectins. FASEB J 1990; 4:2912–2918.
31. Wells V, Mallucci L. Identification of an autocrine negative growth factor: mouse β-galactoside binding protein is a cytostatic factor and cell growth regulator. Cell 1991; 64:91–97.
32. Roff CF, Wang JL. Endogenous lectins from cultured cells. J Biol Chem 1983; 58:10657–10663.
33. Agrwal N, Wang JL, Voss PG. Carbohydrate-binding protein 35. J Biol Chem 1989; 264:17236–17242.
34. Moutsatsos IK, Davis JM, Wang JL. Endogenous lectins from cultured cells: subcellular localization of carbohydrate-binding protein 35 in 3T3 fibroblasts. J Cell Biol 1986; 102:477–483.
35. Moutsatsos IK, Wade M, Schindler M, Wang JL. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. Proc Natl Acad Sci USA 1987; 84:6452–6456.
36. Cowles EA, Moutsatsos IK, Wang JL, Anderson RL. Expression of carbohydrate binding protein 35 in human fibroblasts: comparisons between cells with different proliferative capacities. Exp Gerontol 1989; 24:577–585.
37. Jia S, Wang JL. Carbohydrate binding protein 35. J Biol Chem 1988; 263:6009–6011.
38. Laing JG, Wang JL. Identification of carbohydrate binding protein 35 in heterogeneous nuclear ribonucleoprotein complex. Biochemistry 1988; 27:5329–5334.
39. Oda Y, Leffler H, Sakakura S, Kasai KI, Barondes SH. Human breast carcinoma cDNA encoding a galactoside-binding lectin homologous to mouse Mac-2 antigen. Gene 1991; 99:279–283.
40. Arumugham RG, Hsieh TCY, Tanzer ML, Laine RA. Structures of the asparagine-linked sugar chains of laminin. Biochim Biophys Acta 1986; 883:112–126.
41. Knibbs RN, Perini F, Goldstein IJ. Structure of the major concanavalin A reactive oligosaccharides of the extracellular matrix component laminin. Biochemistry 1989; 28:6379–6392.
42. Zhu BCR, Fisher SF, Pande H, Calaycay J, Shively JE, Laine RA. Human placental (fetal) fibronectin: increased glycosylation and higher protease resistance than plasma fibronectin. J Biol Chem 1984; 259: 3962–3970.
43. Zhu BCR, Laine RA. Polylactosamine glycosylation on human fetal placental fibronectin weakens the binding affinity of fibronectin to gelatin. J Biol Chem 1985; 260:4041–4045.
44. Takamoto M, Endo T, Isemura M, Kochibe N, Kobata A. Structures of asparagine-linked oligosaccharides of human placental fibronectin. J Biochem 1989; 105:742–750.
45. Takamoto M, Endo T, Isemura M, Yamaguchi Y, Okamura K, Kochibe N, Kobata A. Detection of bisected biantennary form in the asparagine-linked oligosaccharides of fibronectin isolated from human term amniotic fluid. J Biochem 1989; 106:228–235.
46. Cossu G, Warren L. Lactosaminoglycans and heparan sulfate are covalently bound to fibronectins synthesized by mouse stem teratocarcinoma cells. J Biol Chem 1983; 258:5603–5607.
47. Zhou Q, Cummings RD. The S-type lectin from calf heart tissue binds selectively to the carbohydrate chains of laminin. Arch Biochem Biophys 1990; 281:27–35.
48. Zhou Q, Cummings RD. L-14 lectin recognition of laminin and its promotion of *in vitro* cell adhesion. Arch Biochem Biophys 1993; 300:6–17.
49. Catt JW, Harrison FL. Selective association of an endogenous lectin with connective tissues. J Cell Sci 1985; 73:347–359.
50. Catt JW, Harrison FL, Carleton JS. Distribution of an endogenous β-galactoside-specific lectin during fetal and neonatal rabbit development. J Cell Sci 1987; 87:623–633.
51. Kobiler D, Barondes SH. Lectin activity from embryonic chick brain, heart, and liver: changes with development. Dev Biol 1977; 60:326–330.
52. Cook GMW, Zalik SE, Milos N, Scott V. A lectin which binds specifically to β-D-galactoside groups is present at the earliest stages of chick embryo development. J Cell Sci 1979; 38:293–304.
53. Powell JT, Whitney PL. Postnatal development of rat lung. Biochem J 1980; 188:1–8.
54. Barondes SH, Haywood-Reid PL. Externalization of an endogenous chicken muscle lectin with in vivo development. J Cell Biol 1981; 91: 568–572.
55. Beyer EC, Barondes SH. Quantitation of two endogenous lactose-inhibitable lectins in embryonic and adult chicken tissues. J Cell Biol 1982; 92:23–27.
56. Harrison FL, Fitzgerald JE, Catt JW. Endogenous β-galactoside-specific lectins in rabbit tissues. J Cell Sci 1984; 72:147–162.
57. Regan LJ, Dodd J, Barondes SH, Jessell TM. Selective expression of endogenous lactose-binding lectins and lactoseries glycoconjugates in subsets of rat sensory neurons. Proc Natl Acad Sci USA 1986; 83: 2248–2252.
58. Levi G, Teichberg VI. Patterns of expression of a 15K β-D-galactoside-specific lectin during early development of the avian embryo. Development 1989; 107:909–921.
59. Gitt MA, Barondes SH. Genomic sequence and organization of two members of a human lectin gene family. Biochemistry 1991; 30:82–89.
60. Chiariotti L, Wells V, Bruni CB, Mallucci L. Structure and expression of the negative growth factor mouse β-galactoside binding protein gene. Biochim Biophys Acta 1991; 1089:54–60.
61. Cherayil BJ, Chaitovitz S, Wong C, Pillai S. Molecular cloning of a human macrophage lectin specific for galactose. Proc Natl Acad Sci USA 1990; 87:7324–7328.
62. Woo HJ, Shaw LM, Messier JM, Mercurio AM. The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). J Biol Chem 1990; 265:7097–7099.
63. Sato S, Hughes RC. Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin. J Biol Chem 1992; 267:6983–6990.
64. Knibbs RN, Agrwal N, Wang JL, Goldstein IJ. Carbohydrate binding protein 35. J Biol Chem 1993; 268:14940–14947.
65. Massa SM, Cooper DNW, Leffler H, Barondes SH. L-29, and endogenous lectin, binds to glycoconjugate ligands with positive cooperativity. Biochemistry 1993; 32:260–267.
66. Woo HJ, Lotz MM, Jung JU, Mercurio AM. Carbohydrate-binding protein 35 (Mac-2), a laminin-binding lectin, forms functional dimers using cysteine 186. J Biol Chem 1991; 266:18419–18422.
67. Brassart D, Kolodziejczyk E, Granato D, Woltz A, Pavillard M, Perotti F, Frigeri L, Liu FT, Borel Y, Neeser JR. An intestinal galactose-specific lectin mediates the binding of murine IgE to mouse intestinal epithelial cells. Eur J Biochem 1992; 203:393–399.
68. Leenan PJM, Jansen AMAC, van Ewijk W. Murine macrophage cell lines can be ordered in a linear differentiation sequence. Differentiation 1986; 32:157–164.
69. Nibbering PH, Leijh PCJ, van Furth R. Quantitative Immunocytochemical characterization of mononuclear phagocytes. Cell Immunol 1987; 105:374–385.
70. Robertson MW, Albrandt K, Keller D, Liu FT. Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. Biochemistry 1990; 29:8093–8100.
71. Shaw LM, Messier JM, Mercurio AM. The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the $\alpha_6\beta_1$ integrin. J Cell Biol 1990; 110:2167–2174.
72. Sato S, Hughes RC. Control of Mac-2 surface expression on murine macrophage cell lines. Eur J Immunol 1994; 24:216–221.
73. Sato S, Hughes RC. Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. J Biol Chem 1994; 269:4424–4430.
74. Frigeri LG, Liu FT. Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages. J Immunol 1992; 148:861–867.
75. Liu FT. Molecular biology of IgE-binding protein, IgE binding factors, and IgE receptors. Crit Rev Immunol 1990; 10:289–306.
76. Truong MJ, Gruart V, Liu FT, Prin L, Capron A, Capron M. IgE-binding molecules (Mac-2/eBP) expressed by human eosinophils. Implication in IgE-dependent eosinophil cytotoxicity. Eur J Immunol 1993; 23:3230–3235.
77. Tachi C, Tachi S, Knyszynski A, Lindner HR. Possible involvement

of macrophages in embryo-maternal relationships during ovum implantation in the rat. J Exp Zool 1981; 217:81–92.
78. Tachi C, Tachi S. Macrophages and Implantation. Ann NY Acad Sci 1986; 476:158–182.
79. Hunt JS. Immunologically relevant cells in the uterus. Biol Reprod 1994; 50:461–466.
80. Brandon JM. Leucocyte distribution in the uterus during the preimplantation period of pregnancy and phagocyte recruitment to sites of blastocyst attachment in mice. J Reprod Fertil 1993; 98:567–576.
81. Brandon JM. Macrophage distribution in decidual tissue from early implantation to the periparturient period in mice as defined by the macrophage differentiation period in mice as defined by the macrophage differentiation antigens F4/80, macrosialin and the type 3 complement receptor. J Reprod Fertil 1995; 103:9–16.
82. Linnemeyer PA, Pollack SB. Murine granulated metrial gland cells at uterine implantation sites are natural killer lineage cells. J Immunol 1991; 147:2530–2535.
83. Parr EL, Young LHY, Parr MB, Young JDE. Granulated metrial gland cells of pregnant mouse uterus are natural killer-like cells that contain perforin and serine esterases. J Immunol 1990; 145:2365–2372.