Expression of mRNA encoding decidualization-associated protein, a variant of acute-phase α_2 macroglobulin, by rat uterine tissues during pregnancy and pseudopregnancy

G. Correia da Silva¹, N. Teixeira¹, J. H. Pringle² and S. C. Bell³

¹Department of Biochemistry, Faculty of Pharmacy and Centre of Experimental Cytology, Oporto University, Anibal Cunha st, 4000 Porto, Portugal; ²Department of Pathology; and ³Department of Obstetrics and Gynaecology, The Medical School, University of Leicester, Leicester, LE2 7LX, UK

The patterns of expression of mRNA encoding decidualization-associated protein (DAP) in the rat uterus from early, mid-, and late stages of pregnancy were examined by *in situ* hybridization to provide an insight into the function of DAP during pregnancy. Parallel studies were performed on rat uteri during the oestrous cycle and oil-induced deciduomata. DAP transcripts were absent in virgin uteri and during the day of implantation (day 5), low in early gestation tissues (days 8, 9) and high during midgestation (days 10–14). Expression of mRNA encoding DAP was first detected on day 8 of pregnancy in decidual cells of the lateral decidual glycogen wing areas. On day 9, mRNA encoding DAP was found in the same area as on day 8 as well as in the outer layer of cells in the antimesometrial decidua adjacent to the myometrium termed the fibrinoid capsula. On days 11 and 12, expression appeared in the decidual cells of the mesometrial decidua, and was particularly strong around the ectoplacental cone and blood vessels that were invaded by the trophoblast. By day 13 and throughout the remainder of pregnancy, expression occurred in the mesometrial decidua and metrial gland. No signal was observed in granulated metrial gland cells. mRNA encoding DAP was also found in the smooth circular muscle coat adjacent to the mesometrial decidua. A similar pattern and cellular localization of expression of mRNA encoding DAP expression was detected during the development of the deciduomata, artificially induced in the absence of a conceptus. The spatial and temporal patterns of expression correlated with a possible role for DAP in mediating fetal–maternal interactions and the establishment of the placental structure.

Introduction

Decidualization in the rat occurs in response to the implanting embryo or to artificial stimuli. The decidual reaction involves the rapid and spatially coordinated progression of proliferation and differentiation of uterine fibroblast-like stromal cells (De Feo, 1967; Finn, 1971; Bell, 1983). The morphological changes are accompanied by metabolic modifications and the synthesis of new proteins. The main secretory product of rat decidua is a glycoprotein of high molecular mass termed ‘decidualization-associated protein’ (DAP) (Bell, 1979a). DAP shares physico-chemical properties and immunochemical identity with rat acute-phase serum protein α_2 -macroglobulin (Bell, 1979b), and further biochemical studies have demonstrated that DAP is a charge variant of α_2 -macroglobulin (Teixeira and O’Grady, 1992).

Rat α_2 -macroglobulin is a member of the high molecular mass glycoprotein α -macroglobulin family (Sottrup-Jensen, 1989). In many species α_2 -macroglobulin exhibits highly conserved

sequences except for the so-called ‘bait region’. Human α_2 -macroglobulin is a general proteinase inhibitor that interacts with all classes of proteinase (Barrett and Starkey, 1973), binds cytokines and growth factors (reviewed in LaMarre *et al.*, 1991) and is produced by the endometrium during the menstrual cycle (Sayegh *et al.*, 1995).

α_2 -Macroglobulin is a tetramer glycoprotein composed of identical subunits (180 kDa), each one containing a specific sequence, the ‘bait region’, that is susceptible to proteolytic cleavage. This cleavage causes a conformational change of the molecule and the entrapment of the proteinase by a mechanism described by the ‘trap’ hypothesis (Barrett and Starkey, 1973). The conformational change exposes the receptor-binding domain (Gonias *et al.*, 1983) that is recognized by specific receptors in various cell types including human syncytiotrophoblast (Jensen *et al.*, 1988).

Specific mRNA encoding α_2 -macroglobulin has been identified in decidual tissue (Hayashida *et al.*, 1986) and its concentrations during pregnancy reflect the synthesis of DAP *in vitro* (Bell, 1979a). mRNA encoding α_2 -macroglobulin has been detected in decidual cells by northern blot analysis; the signal is strongly

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expressed in the decidua from days 10 to 15 of gestation (Fletcher *et al.*, 1988). Gu *et al.* (1992) concluded that DAP is secreted exclusively by the mesometrial decidua. Thomas (1993) studied expression of mRNA encoding α_2 -macroglobin during the postimplantation period until day 13 of pregnancy by *in situ* hybridization. However, the distribution was only examined at low power and the extent to which maternal uterine tissues transcribe the gene has not been fully defined.

The aim of the present study was to examine the distribution of mRNA encoding DAP in uteri of virgin and pregnant rats during several stages, starting on day 5 of pregnancy and continuing to the end of pregnancy.

Materials and Methods

Animals

Wistar rats (200–250 g) were kept under standard conditions of 14 h light:10 h dark. Food and water were always available. Female rats were mated, and the day on which spermatozoa were found in the morning vaginal smear was designated day 1 of pregnancy. For induction of decidualomata, the ovarian end of each uterine horn was exteriorized through an incision in the flank and 0.5 ml of arachis oil injected into the uterine lumen under Fluthane anaesthesia.

Implantation sites on day 6 and day 7 of pregnancy were visualized by injecting 1% (w/v) Pontamine Sky Blue (BDH Chemicals, Poole, Dorset; Psychoyos, 1960) into the lateral tail vein 15 min before autopsy. Animals were killed by cervical dislocation, the uterine horns dissected out and fixed immediately in 10% (v/v) formal saline for 24 h. Haematoxylin and eosin were used for the study of general morphology.

In situ hybridization methods

Preparation of tissues. Tissues were fixed in 10% (v/v) formal saline and processed in paraffin wax. The block was orientated to enable the implantation sites to be sectioned either transversely or longitudinally. Serial sections (4 μ m) were cut through each implant site in the area containing the embryo and mounted on slides coated with 3-aminopropyltriethoxysilane (Sigma Chemical Co, St. Louis, MO). The slides were dewaxed in xylene and hydrated through graded alcohols into DEPC (diethylpyrocarbonate, Sigma Chemical Co)-treated water.

Pretreatments. Pretreatments were carried out as described by Pringle *et al.* (1990). Briefly, the slides were immersed into $2 \times$ SSC buffer (SSC is 150 mmol NaCl l^{-1} , 15 mmol trisodium citrate l^{-1} , pH 7.0) at 70°C for 10 min and transferred to DEPC-water at ambient temperature for 5 min. The sections were then digested in proteinase K (Boehringer Mannheim, Mannheim) solutions in 50 mmol Tris-HCl l^{-1} , pH 7.6, at concentrations ranging from 5 to 10 μ g ml^{-1} and incubated at 37°C for 1 h in a humidity chamber. When required RNase pretreatment controls were carried out at this stage. RNase

type 1A (Sigma Chemical Co) digests were performed at 100 μ g ml^{-1} , at 37°C for 1 h. Finally the sections were washed in DEPC-water and post-fixed in 0.4% (w/v) paraformaldehyde, 0.1 mol PBS l^{-1} /DEPC at 4°C in precooled solutions.

Preparation of probes. Probes were designed to hybridize to the transcripts of mRNA encoding α_2 -macroglobin and to avoid likely regions of homology amongst members of the α -macroglobulin family. The probes were targeted against three different portions of the less well conserved 'bait region' transcript. Three probes of 30 bases were synthesized in the Department of Biochemistry, University of Leicester. Care was taken to avoid making probes that would hybridize to areas of predicted secondary mRNA structure. Synthetic oligonucleotides were synthesized by phosphoramidite chemistry (Beaucage and Caruthers, 1981) using a DNA synthesizer model 380A (Applied Biosystems, Foster City, CA). Base additions were made using *o*-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidites (Cruachem, Glasgow) as described by McBride *et al.* (1987). A 5' primary amine group was then added by reaction with Aminolink2 (Applied Biosystems) diluted in 0.1 mol dimethylaminopyridine l^{-1} in anhydrous acetonitrile. After deprotection, the crude oligomers were desalted, ethanol precipitated, evaporated to dryness, dissolved in DEPC ultra-pure water and quantified by measuring the absorbance of samples at 260 nm.

Oligonucleotide labelling. The labelling reaction was carried out on cocktails of three oligonucleotides:

5'-GCA CTG GCG AGT ACA GGA TTC CAT TAA TGT-3';
5'-GAC TTT GGG TTT ACG GAT GTT CGA GTT GGT-3'; and
5'-GGA GAC TCT GAA GAC TCT AGA AAA GCG TCC-3'.

Oligonucleotides were labelled at the 3' end using the TdT reaction as described by Pringle *et al.* (1989) to add a homopolymer tail of digoxigenin-11-dUTP (Boehringer Mannheim). This reaction is based on the improved procedure for homopolymer tailing using TdT as described by Deng and Wu (1981). The labelled oligonucleotides were purified by passage through a TE equilibrated Sephadex G50 spun column (Pharmacia Biothec, Uppsala) based on the procedure described by Sambrook *et al.* (1989) and stored at -20°C .

Hybridization and post-hybridization washing. The hybridization and post-hybridization conditions were as described by Pringle *et al.* (1990). The sections were drained, excess fluid was removed and the slides were then covered in 50 μ l of pre-hybridization buffer containing (final concentration) 0.6 mol NaCl l^{-1} ; $1 \times$ PE (50 mmol Tris-HCl l^{-1} , pH 7.5, 0.1% (w/v) sodium pyrophosphate, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll, 50 mmol EDTA l^{-1}); 10% (w/v) dextran sulfate; 150 μ g salmon sperm DNA ml^{-1} ; 30% (v/v) formamide. The sections were pre-hybridized for 1 h at 37°C in a humid chamber in a hot-air incubator. After pre-hybridization the slides were drained and the sections were covered with hybridization buffer containing labelled probe at 500 ng ml^{-1} . The sections were then covered with siliconized coverslips (dimethyldichlorosilane, Sigma Chemical Co) to ensure even coverage with the hybridization solution. The slides were then

returned to the humid chambers and were incubated at 37°C overnight.

After hybridization, the coverslips were removed from the sections by transferring the slides to $2 \times \text{SSC}/30\%$ (v/v) formamide pre-warmed to 37°C. The sections were then washed with $2 \times \text{SSC}/30\%$ (v/v) formamide at 37°C (2×10 min). Finally the sections were washed in blocking solution consisting of 3% (w/v) 0.1% (v/v) Triton X-100 in TBS (50 mmol Tris-HCl l^{-1} , pH 7.6, 0.15 mol NaCl l^{-1}) at room temperature for 5 min.

Detection. A direct detection method using anti-digoxigenin alkaline phosphatase conjugate (Boehringer Mannheim) was used. The sections were incubated with sheep anti-digoxigenin-AP, diluted in blocking solution, in a moist chamber at room temperature for 30 min. They were then washed in TBS ($\times 2$) and in substrate buffer (0.1 mol Tris l^{-1} pH 9.0, 0.1 mol NaCl l^{-1} , 50 mmol MgCl_2 l^{-1}). Alkaline phosphatase activity was demonstrated using the 5-bromo-4-chloro-3-indolyl phosphate (BCIP enzyme substrate; Sigma Chemical Co) and nitroblue tetrazolium (NBT chromogen; Sigma Chemical Co) method as described by Pringle *et al.* (1989). For optimum detection, sections were incubated in the demonstration solution overnight and then washed in ultra-pure water and mounted in Apathy's medium.

Controls. A known positive control (fetal liver) and a negative control where the probe was not included in the hybridization stage were included in each run. RNase pretreatments were carried out to show that RNA, not DNA, was detected. This abolished the signal in all cases.

Extraction of RNA and northern blot analysis

Total RNA was isolated from fresh tissues (decidual tissue from samples taken on day 12 of pregnancy and adult liver) using a mixture of 4 mol guanidine thiocyanate l^{-1} , 20 mmol sodium acetate l^{-1} (pH 5.2), 0.1 mmol dithiothreitol l^{-1} and 0.5% (w/v) *N*-lauroylsarcosine (Chomczynski and Sacchi, 1987). RNA was quantified by measuring the absorbance of samples at 260 nm. Thirty micrograms of total RNA was denatured with glyoxal and dimethyl sulfoxide (Sambrook *et al.*, 1989) and fractionated by electrophoresis through a 1.5% (w/v) agarose gel. RNA was transferred to a nylon membrane. The blot was prehybridized for 5 h at 65°C in a solution that comprised $5 \times \text{SSC}$, $5 \times$ Denhardt's reagent (polyvinylpyrrolidone, BSA, Ficoll 400), 2.5% (w/v) SDS, 200 μg denatured salmon sperm DNA ml^{-1} . The blot was then hybridized overnight with ^{32}P -labelled probe in the same solution. After hybridization, the blot was washed twice in $2 \times \text{SSC}$, 0.1% (w/v) SDS for 3 min at room temperature and exposed to Kodak XAR-2 film (Rochester, NY) for 48 h at -70°C .

The probe used for hybridization was the cocktail of the three oligonucleotides that had been used for the *in situ* reaction, 3' end-labelled with terminal deoxynucleotidyl transferase (TdT).

Results

The specificity of the probe used, which corresponded to segments of the 'bait region', was confirmed on northern blot

Fig. 1. Northern blot analysis of total RNA (30 μg) extracted from rat decidual tissue on day 12 of pregnancy (lane 2). A single 5.2 kb band, corresponding to α_2 -macroglobin, was detected. Lane 1: total RNA from adult rat liver (30 μg). Exposure time 48 h.

analysis of total RNA obtained from decidual tissue on day 12 of pregnancy. A single 5.2 kb band was detected that corresponded to the size of mRNA for authentic α_2 -macroglobin from other tissues (Fig. 1). Fetal liver strongly expresses mRNA encoding α_2 -macroglobin (Kodelja *et al.*, 1986) and was used as a positive control where the fetal liver could be localized within the fetus. RNase digestion abolished the signal on all the days studied (Fig. 3d) indicating the specificity of the signal.

During the oestrous cycle, mRNA encoding DAP was not detected in any uterine tissue by *in situ* hybridization (not shown). On day 5 of pregnancy implantation occurred in the anti-mesometrial endometrium and on days 6–7 the primary decidual zone develops from the endometrial stromal cells surrounding the implanted blastocyst in the antimesometrial pole. No signal was detected on days 5, 6 and 7 of pregnancy in any tissue (not shown).

By day 8 the decidual reaction that had begun anti-mesometrially in the connective tissue stroma of implantation sites was present in a large area of the antimesometrial decidua, whereas the mesometrial endometrium was in an early stage of decidualization. A relatively light expression of mRNA encoding DAP could be detected in the cytoplasm of mesometrial decidual cells, forming two patches of signal on either side of the embryo. In the antimesometrial region, a very light staining was now detected in the antimesometrial decidua lateral and adjacent to the myometrium layer in a thin area that has not decidualized, probably the area described as the fibrinoid capsule by Bulmer and Dickson (1961).

Fig. 2. Transverse sections of uteri taken from rats at days 9 (a, b), 10 (c, d) and 11 (e, f) of pregnancy. The ectoplacental cone invades the mesometrial decidua in days 10 and 11. (a, c, e) Sections stained with haematoxylin and eosin. (b, d, f) Expression of mRNA encoding decidualization-associated protein is seen in lateral decidual cells, fibrinoid capsule and smooth muscle layer. MD: mesometrial decidua; LD: lateral decidual cells; AMD: antimesometrial decidua; FC: fibrinoid capsule; C: conceptus; MC: circular muscle layer; MG: metrial gland; Tr: trophoblast. Scale bars represent 1 mm. Squares indicate regions shown at higher magnification in Fig. 3.

By day 9, the pattern of expression, although the same as on day 8, had become more prominent. The signal was intense in the decidual cells lateral to the conceptus (Fig. 2b). The decidual cells in the mesometrial pole are essentially negative for expression of mRNA encoding DAP; however, a layer of cells adjacent to the circular muscle coat was positive in the mesometrial region (Fig. 2b).

By day 10, the antimesometrial decidua was regressing, the mesometrial decidua was continuing to develop and the ectoplacental cone had started to invade maternal tissues (Fig. 2c). In the antimesometrial area, the decidual cells remained negative and the non-decidualized stromal cells close to the myometrium layer continued to express the signal strongly (Fig. 2d). In this area the myometrium was negative. In contrast, in the mesometrial region, the thinning circular muscle layer and adjacent cells in the endometrium exhibited a strong expression. An intense signal was found in the lateral mesometrial decidua, the glycogen cell wing area, although the signal decreased in the direction of the central mesometrial region. There was no expression of mRNA encoding DAP in the central area of the mesometrial decidua, even in the area being invaded by the trophoblast of the ectoplacental cone (Fig. 3a).

On day 11, the expression in the mesometrial decidua remained the same as on day 10. However, the central region of the mesometrial decidua had become positive. In this region, the myometrium exhibited a strong signal in the layer close to the non-decidualized stromal cells (Fig. 2f).

On day 12, the definitive placenta has formed in the mesometrial region, and invasion of the central mesometrial artery with endovascular trophoblast occurred (Fig. 4a). mRNA encoding DAP was identified in all the mesometrial decidual cells, the positivity being particularly strong near the central area, where the trophoblast cells have invaded the maternal tissue, and around the trophoblast containing maternal blood vessels. However, no transcripts were detected either in cytotrophoblasts or in placental giant trophoblast cells. mRNA encoding DAP was also detected in some cells within the metrial gland region (Fig. 4b).

On day 13, all the mesometrial area was stained, although, in the lateral areas the expression was not as intense as in day 12 (Fig. 4d). The expression was most intense in cells surrounding the invaded blood vessels which now extended into the metrial gland (Fig. 4f). Fibroblast cells positive for mRNA encoding DAP formed a sheath with negative metrial gland cells around an artery containing trophoblastic cells. The stretched inner circular muscle layer was very positive. The metrial gland area presented a very strong signal.

On day 14, the thinning circular muscle layer and metrial gland continued to show an intense signal (Fig. 5b). The decidua basalis formed from the mesometrial decidua was positive, in contrast to the negativity of the adjacent placenta. The giant trophoblast cell layer remained distinctly negative. In the metrial gland all the cells were expressing mRNA encoding DAP but granulated metrial gland cells were distinctly negative (Fig. 5e).

From day 14 onwards, and until the end of pregnancy, mRNA encoding DAP continued to be expressed in the mesometrial decidua throughout its regression. The pattern of expression on day 19 of pregnancy is shown (Fig. 5d).

The pattern of expression observed in the deciduomata was essentially the same as in pregnancy (Fig. 3c).

Discussion

The results presented here demonstrate that the expression of the pregnancy-associated variant of α_2 -macroglobin, DAP, is specific in terms of the period of pregnancy, cell type and tissue localization with respect to the conceptus, and the development of regions of the uterus associated with decidualization. The results correlate well with previous *in vitro* estimates of DAP synthesis at the protein level by the 'implantation site' during pregnancy (Bell, 1979a, b). Studies with artificially induced deciduomata in pseudopregnant animals indicated that this pattern of expression was programmed and reflected morphogenesis of the uterine decidua.

There was no detectable expression in the cells of the antimesometrial decidua throughout its development. Although it is accepted that the embryo implants into this region on days 5–9, this early period does not appear to represent an 'invasive' type of implantation, since the antimesometrial decidua develops and grows mesometrially around the embryo to form a 'crypt', with the embryo being essentially passive (Welsh and Enders, 1983). The lack of expression of α_2 -macroglobin by these cells may indicate that the embryo is not invasive during this phase of implantation and does not necessitate the production of antiproteases by local maternal tissue.

However, expression of mRNA encoding α_2 -macroglobin was detected on days 9 and 10 in stromal fibroblastic cells of the antimesometrial endometrium between the outer limit of the characteristic antimesometrial decidual cells and the undifferentiated basal endometrium adjacent to the circular muscle coat of the myometrium. This corresponds to the layer destined to form the 'fibrinoid capsula' (Bulmer and Dickson, 1961). This basal layer of cells appear fibroblastic in nature and it is possible that expression of α_2 -macroglobin is initially induced in these cells by local stretching, and maintained subsequently by involution of the decidual cells within the antimesometrial decidua during the remodelling in this zone. DAP or decidual α_2 M synthesis is specific to the mesometrial decidua of the deciduomata of pseudopregnancy (Jayatilak *et al.*, 1989; Gu *et al.*, 1992); however, detection of induced expression of mRNA encoding α_2 -macroglobin in a population of non-decidualized cells associated with the antimesometrial decidua or deciduomata does not support such specificity.

On days 8–10 expression of mRNA encoding DAP was detected in the lateral mesometrial decidua, the so-called 'glycogen wing area' through which maternal blood drains from the implantation site into venous sinusoids (Enders and Welsh, 1993). During the development of this area, although margination of polymorphonuclear cells is observed, they fail to migrate into the surrounding lateral decidua. It would be interesting to determine whether α_2 -macroglobin has an inhibitory role in this phenomenon given its ability to bind cytokines (Correia da Silva *et al.*, 1993, 1996).

While the lateral decidua may provide a 'circular jacket' positive for α_2 -macroglobin, mRNA encoding α_2 -macroglobin is absent in the central area of the mesometrial decidua

Fig. 3. Transverse sections of uteri and deciduomata taken from rats at days 10 and 11 of pregnancy. (a) Higher magnification of the region depicted in square of Fig. 2d (day 10). (b) Higher power of the region depicted in square of Fig. 2f (day 11). (c) Expression of mRNA encoding decidualization-associated protein in a transverse section of deciduomata. (d) Negative control: RNase pretreated section of uterus at day 10 of pregnancy. The signal was abolished. The region shown is the antimesometrial region and fibrinoid capsule. LD: lateral decidual cells; MD: mesometrial decidua; AMD: antimesometrial decidua; FC: fibrinoid capsule; C: conceptus; MC: circular muscle layer; Tr: trophoblast. Scale bars represent (a) 500 μ m, (b), 100 μ m and (c, d) 1 mm.

Fig. 4. Transverse sections of uteri taken from rats at day 12 (a, b, e) and day 13 (c, d, f). (a, c) Sections stained with haematoxylin and eosin. (b, d) mRNA encoding DAP is seen over all the mesometrial decidua, muscle cell layer and also to a small extent in the metrial gland region. (e, f) Higher magnification pictures of sections indicated by squares in (b) and (d), respectively. MD: mesometrial decidua; MG: metrial gland; MC: circular muscle layer; Tr: cytotrophoblast; BV: blood vessel; P: placenta. Scale bars represent (a, b, c, d) 1 mm and (e, f) 100 μ m.

Fig. 5. Transverse sections of uteri taken from rats at days 14 (a, b, e) and 19 (c, d, f) of pregnancy. (a, c) Sections stained with haematoxylin and eosin. (b) Expression of mRNA encoding decidualization-associated protein (DAP) in midgestational decidua and metrial gland. Expression is strong in the circular muscle layer and metrial gland. Subjacent to the stretched circular muscle layer is the decidua basalis and then the placenta. The placenta shows no signal for mRNA encoding DAP. The square indicates the region depicted in (e). (d) Expression of mRNA encoding DAP in decidua from rats at day 19 of pregnancy. The placenta shows no signal for mRNA encoding DAP. There is strong expression of mRNA encoding DAP in the metrial gland. (e) Granulated metrial gland cells (GMG) (arrows) are negative for mRNA encoding DAP. These cells contain prominent intracellular granules. (f) Transverse section (day 19) in which the fetal liver can be seen. The fetal liver (arrow) strongly expresses the signal for mRNA encoding DAP. MD: mesometrial decidua; MG: metrial gland; MC: circular muscle layer; P: placenta; BV: blood vessel; DB: decidua basalis. Scale bars represent (a, b, c, d) 100 μ m and (f) 2 mm.

underlying the implantation chamber. It is of interest to note that days 10–12 represent the truly invasive phase of implantation whereby the ectoplacental cone of the conceptus invades into this central region deficient in mRNA encoding α_2 -macroglobin. Any proteinase-mediated trophoblastic invasion may be free from the potent proteinase inhibitory effects of locally produced α_2 -macroglobin (Teixeira *et al.*, 1991).

On days 11 to 12, trophoblast invasion of the central mesometrial artery occurs. Within the mesometrial decidua expression of mRNA encoding α_2 -macroglobin was only associated with the mesometrial decidual cells. Expression was most intense in the mesometrial decidual cells clustered as a sheath around the artery. However a negative, inner area adjacent to the artery, and composed of granulated metrial gland cells did not express mRNA encoding α_2 -macroglobin. From day 13 a similar relationship was detected within the metrial gland, where fibroblastic cells positive for mRNA encoding α_2 -macroglobin formed a sheath around an artery containing trophoblastic cells encapsulated by a metrial gland cell layer negative for mRNA encoding α_2 -macroglobin. The function of granulated metrial gland cells remains unclear. However, their cytoplasmic granules are known to contain serine esterases and perforin (Parr *et al.*, 1990), cytolytic proteins found in the highly cytotoxic NK and T lymphocytes and, through their discharge, may be involved in changes within the arterial wall facilitating trophoblast invasion. It is possible that metrial gland fibroblastic cells share a similar phenotype and function to mesometrial decidual cells in that they, via a common expression of α_2 -macroglobin, fulfil a function in controlling the activity of granulated metrial gland cells.

As the definitive haemochorial placenta develops and the fetus continues to grow, the circular muscle coat beneath the placenta appears to be stretched and dissociates into clusters of circular muscle cells, and the central area of the coat is breached. Expression of mRNA encoding α_2 -macroglobin by these smooth muscle cells was very intense and persisted throughout pregnancy suggesting that at this site, smooth muscle cells may be induced to express α_2 -macroglobin or that these cells undergo differentiation to stromal fibroblastic cells.

The cell-specific expression of the decidua-derived variety of α_2 -macroglobin, DAP, detected in this study indicates that the interrelationships between cell types in the uterus should be re-examined. Antimesometrial decidual cells, in contrast to other decidual cell types, do not produce α_2 -macroglobin and indeed appear unique in that they also exhibit different ultrastructural and structural features to any other decidual cell type. Lateral decidual cells and mesometrial decidual cells are characterized by their high expression of mRNA encoding α_2 -macroglobin. However, it appears that fibroblastic cells within the metrial gland also possess this property and exhibit similar expression when juxtaposed to arteries and granulated metrial gland cells, as with mesometrial decidual cells in the mesometrial decidua. Given that fibroblastic cells in the basal layer of the antimesometrial decidua between the muscle coats of the mesometrial myometrium, the metrial triangle, and the circular muscle cells of the vascularised mesometrial myometrium all exhibit inducible expression of α_2 -macroglobin, this raises questions as to their ontogenic relationships.

In animals with deciduomata, the pattern of expression of mRNA encoding α_2 -macroglobin was identical to that in the decidua of pregnancy in terms of cellular localization. Synthesis of decidua α_2 -macroglobin therefore reflects the pattern of temporal and spatial growth and differentiation of the decidua, whether the conceptus is present or not, and supports the contention that this phenomenon is hormonally programmed and is subsequently controlled by local interactions (Bell, 1983). The presence of the growing conceptus may only provide further pressure and stretching of the uterine wall and further induce expression of α_2 -macroglobin at stretch-induced sites.

It has been proposed that one function of the decidua is to control trophoblastic invasion and is in part mediated by local production of α_2 -macroglobin (Bell, 1983). The pattern of the expression of α_2 -macroglobin, both spatially and temporally, supports a possible controlling influence in permitting or directing trophoblastic invasion into the central mesometrial decidua containing the mesometrial artery. By virtue of its ability to inhibit a wide range of proteinases (Teixeira *et al.*, 1991) and its ability to bind to a variety of growth factors and cytokines (Correia da Silva *et al.*, 1993, 1996) α_2 -macroglobin could mediate a controlling role in the invasion of the trophoblast and of the granulated metrial gland cells. Whether the pattern of DAP protein expression and its deposition follows a similar pattern waits further study.

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