Expression of tissue type and urokinase type plasminogen activators as well as plasminogen activator inhibitor type-1 and type-2 in human and rhesus monkey placenta

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ABSTRACT

The distribution of mRNAs and antigens of tissue type (t) and urokinase type (u) plasminogen activators (PA) plus their corresponding inhibitors, type-1 (PAI-1) and type-2 (PAI-2) were studied in human and rhesus monkey placentae by in situ hybridisation and immunocytochemistry. Specific monkey cRNA and antibodies against human tPA, uPA, PAI-1 and PAI-2 were used as probes. The following results were obtained. (1) All the molecules tPA, uPA, PAI-1 and PAI-2 and their mRNAs were identified in the majority of the extravillous cytotrophoblast cells of the decidual layer between Rohr's and Nitabuch's striae and in cytotrophoblast cells of the chorionic plate, basal plate, intercotyledonary septae and cytotrophoblast cells of the chorionic villous tree. (2) Expression of uPA and PAI-2 was noted in villous trophoblast whereas tPA and PAI-1 were mainly concentrated where detachment from maternal tissue occurs. (3) No expression of tPA, uPA, PAI-1 and PAI-2 was observed in the basal plate endometrial stromal cells, chorionic plate connective tissue cells, septal endometrial stromal cells or villous core mesenchyme. (4) The distribution of probes observed following in situ hybridisation is generally consistent with the immunofluorescence pattern of the corresponding antigens and no significant interspecies differences were noted. It is possible that both decidual and extravillous trophoblast cells of placentae of human and rhesus monkey are capable of producing tPA, uPA, PAI-1 and PAI-2 to differing extents. Coordinated expression of these genes in the tissue may play an essential role in the maintenance of normal placentation and parturition. The differences in distribution we observed are consistent with the suggestion that coordinated expression of tPA and its inhibitor PAI-1 may play a key role in fibrinolytic activity in the early stages of placentation and separation of placenta from maternal tissue at term. On the other hand, uPA with its inhibitor PAI-2 appears mainly to play a role in degradation of trophoblast cell-associated extracellular matrix, and thus may be of greatest importance during early stages of placentation.

Key words: Pregnancy; placentation; trophoblast.

INTRODUCTION

Normal pregnancy and parturition are routinely associated with pronounced changes in the coagulation and fibrinolytic system (Nilsson et al. 1986). The placenta is a key altered source of the coagulation and fibrinolytic components, not only of placental type plasminogen activator inhibitor, PAI-2 (Åstedt et al. 1987; Lecander et al. 1988), but also of the endothelial cell-type plasminogen activator inhibitor, PAI-1 (Ny et al. 1986) as well as the tissue type (Jonasson et al. 1989) and the urokinase type (Hofman et al. 1994) plasminogen activators. Normal fetal growth and development require an adequate supply

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of maternal blood which is delivered to the intervillous spaces of the placenta (Pijnenborg et al. 1980, 1981). This is a complex process associated with uterine-fetal tissue remodelling and vascularisation. Trophoblast plays a central role in the induction of these physiological changes (Hamilton & Boyd, 1960). The conversion of spiral arterioles into uteroplacental arterioles by trophoblast invasion in the early stages of pregnancy is an essential step in placentation and the establishment of an adequate choriodecidual blood flow in normal pregnancy (Meekins et al. 1994). It has been reported that trophoblast implantation, vascular remodelling and maintenance of intervillous blood flow may depend on the regulated production of plasminogen activators and their inhibitors (Fazleabas et al. 1991; Estelles et al. 1994). It is likely that the generation of plasmin by the precisely balanced expression of these activators and inhibitors in trophoblasts and other maternal tissues could lead directly to the degradation of noncollagenous (Saksela, 1985) and collagenous (Mackay et al. 1990; Liotta et al. 1994) components of the extracellular matrix within the placenta. Fisher et al. (1985, 1989) have demonstrated that cultured human trophoblasts degrade extracellular matrix, and these processes can be inhibited by the presence of serine protease inhibitors in vitro (Yagel et al. 1998; Librach et al. 1991), suggesting that PA inhibitors are of equal importance to the activators in these tissues to maintain the balance between proteolytic and anti-proteolytic activity. In this fibrinolytic system tPA is a key enzyme whereas uPA appears to play an essential role in the cell-associated degradation of extracellular matrix during invasive growth of trophoblast (Blasi et al. 1987). PAI-2 is of placental origin (Kawano et al. 1968; Wun & Reich, 1987) and is important for quenching uPA activity, whereas PAI-1 may be produced both by vascular endothelial cells (Loskutoff, 1991) and placental tissue (Lecander et al. 1988) and is the principal physiological inhibitor of tPA (Jorgensen et al. 1989). Both PAI-1 and PAI-2 increase in the peripheral circulation during normal pregnancy (Kruithof et al. 1987; Estelles et al. 1989). It has been suggested that PAI-1 is the key inhibitor in plasma responsible for the rise in PA inhibitory activity in patients with pre-eclampsia (Jorgensen et al. 1987). The decreases in plasma fibrinolytic activity in these patients have been reported to be caused mainly by increased levels of PAI-1 (Wiman et al. 1984), and plasma levels of PAI-1 in these patients are always positively correlated with the severity of placental damage (Estelles et al. 1989). Fibrin deposition or occlusive lesions in placental vasculature and decidual spiral arteries are usually observed in eclamptic patients (Pijnenborg, 1991). Interestingly, the overall depression of fibrinolytic activity in patients with pre-eclampsia appears to centre on inhibition of plasminogen activator rather than inhibition of plasmin itself, because the plasma levels of α_2-antiplasmin (de Boer et al. 1988) as well as the subsidiary inhibitors of plasmin (Gow et al. 1984) showed no significant differences between pre-eclamptic and normal pregnancies. It is therefore suggested that PA (tPA and uPA) and PAI (PAI-1 and PAI-2) of placental origin may be the primary activators and inhibitors responsible for the changes in proteolytic and anti-proteolytic activity during pregnancy. Detailed studies have confirmed the placental production of tPA (Liu et al. 1997), uPA (Martin & Arias, 1982), PAI-1 and PAI-2 (Ye et al. 1987) during pregnancy. A few publications have also addressed the placental content of mRNAs for PAI-1 (Feinberg et al. 1989; Fazleabas et al. 1991; Schneiderman et al. 1991; Estelles et al. 1994) and PAI-2 (Feinberg et al. 1989; Fazleabas et al. 1991). As far as the authors are aware, no data are available to show comparatively the expression of both the antigens and mRNAs for tPA, uPA, PAI-1 and PAI-2 in human and simian placentae. This study was therefore undertaken in an attempt precisely to define the localisation and distribution of both antigens and mRNAs. We have accomplished this for tPA, uPA, PAI-1 and PAI-2 in placentae of human and rhesus monkeys at late stages of gestation. We wished to compare the outcome of this study with the interesting data recently obtained for human and simian amniochorion and decidua that indicate a distribution pattern of synthesis of these compounds important to the physiology of invasion and adhesion of the conceptus during pregnancy and its physiological separation from the mother at parturition (Liu et al. 1998).

MATERIALS AND METHODS

Reagents and antibodies

The riboprobe system for Northern blot analysis was purchased from Promega (Madison, WI); restriction enzymes, Taq polymerase, 4-nitro blue tetrazolium chloride, 5-bromo-4-chloro-3-indolyl-phosphate, anti-digoxygenin-AP F(ab) fragments, the Dig RNA Labelling Kit and blocking reagent for riboprobe of in situ hybridisation were purchased from Boehringer Mannheim (Mannheim, Germany). Normal rabbit and goat sera were obtained from Life Technologies (Paisley, Scotland). Mouse antihuman PAI-2, mouse

antihuman uPA and goat antihuman melanoma tPA were purchased from Biopool (Umeå, Sweden). Rabbit antihuman PAI-1 antibodies were raised by Professor T. Ny (Umeå, University). Goat antirabbit IgG-FITC, goat antimouse IgG-FITC and rabbit antigoat IgG were obtained from Sigma-Aldrich (Dorset, UK).

Tissue preparation

Ten human placentae (gestational age range 5–7 mo) obtained at operation or following spontaneous abortions and 13 trophoblast tissue samples at early stages of gestation (gestational age < 3 mo) were obtained following therapeutic terminations of pregnancy by curettage performed at Beijing Zhongguancun Hospital and immediately returned to the laboratory. All human tissue samples were obtained with informed consent and the approval of local ethical committees. The basal and chorionic plates were gently cut from the placentae into 20 mm × 10 mm strips and frozen either in liquid nitrogen or in hexane/CO_2 slush in disposable paper cups which contained Tissue-Tek O.T.C. embedding compound (Miles Inc. Diagnostic Division Elkhart, IN 46515, USA). Five rhesus monkey placentae with a thin layer of uterine myometrium between d 130–150 of pregnancy were collected following terminations of pregnancy performed for other purposes at Kun-ming Institute of Medical Biology. The basal and chorionic plates were dissected from the placentae and rapidly frozen in the same way as the samples from human placentae. Cryosections (8–12 μm thick) were cut using a Leitz cryostat and melted onto 10-well Multitest slides (Flow Laboratories).

Procedures for indirect immunofluorescence

The sections were fixed in 3% formaldehyde in 0.25 M PBS (pH 7.4), washed twice with PBS and permeabilised in 0.05% Triton X100. The sections were washed again and immersed in 0.25 M L-lysine in PBS for 1 h at 4 °C. After removing excess PBS/lysine from the slides using a vacuum driven suction pump, first stage antibody was added to each experimental well and incubated overnight at 4 °C. After washing in PBS for 1 h at room temperature, the corresponding second stage antibodies which were conjugated to fluorescein isothiocyanate (FITC) were added to all wells and the tissue was incubated for 1 h at 37 °C. The sections were thoroughly washed in PBS and mounted in antiphotobleaching mountant (Citifluor, Agar Aids, Stansted) and sealed with nail varnish. Immunofluorescence specimens were examined under a Zeiss standard epifluorescence microscope (Ockleford et al. 1993). Photographic recording was achieved using a Zeiss MC63 camera attachment and 400 ASA Fujichrome colour slide film.

Confocal epifluorescence microscopy

Sections were examined critically using a Zeiss Axiovert 10 epifluorescence microscope equipped with a Biorad Lasersharp MRC600 confocal laser scanning attachment (Ockleford et al. 1997). Photographic recording from the confocal system was undertaken using, for black and white photography, a Shackman flat screen monitor with camera attachment loaded with Ilford FP4 or, for false colour, a Polaroid Quickprint loaded with E6 colour transparency film such as Polaroid Presentation Chrome. The instrument was calibrated for measurement purposes using a reflective slide-mounted grating (Biorad UK) (Bradbury & Ockleford, 1990).

Synthesis of monkey antisense RNA probes for tPA, uPA, PAI-1 and PAI-2

Polymerase chain reaction (PCR) primers for monkey tPA and PAI-1 fragments were designed using known human cDNA sequences, assuming conservation would ensure efficient hybridisation. They contained restriction endonuclease sites (underlined sequences) for subcloning. In order to design tPA primer pairs for PCR, we used nucleotides 648–667 and 975–994 of the human tPA cDNA sequence (1) (Pennica et al. 1982). For PAI-1 primer pair design we used nucleotides 259–274 and 610–647 of the human PAI-1 cDNA sequence (2) (Ny et al. 1986). For uPA primer pair design we used nucleotides 1211–1231 and 1630–1651 of human uPA cDNA sequence (3) (Verde et al. 1984) and for PAI-2 primer pair design, nucleotides 529–545 and 900–921 of human PAI-2 cDNA sequence (4) (Ny et al. 1989) were used. The primer pairs were as follows: sequence (1) tPA-*Sac*1: 5′ ACT-~~GAGCTC~~CCTGGTGCTACGTGTTTAAGG tPA-*Hind* III: 5′ GCT~~AAGCTT~~ACTGTCTCAGGCCG-CAGGTG 3′; sequence (2) PAI-1 *Sac*1: 5′ CAAG-~~GAGCTC~~ATGGGGCCATGGAACA 3′ PAI-1 *Hind* III: 5′ TAGTT~~AAGCTT~~GTTGGTCTGTGA-GCCATCATGGG 3′; sequence (3) uPA: 5′ TGC-TCACCACAACGACATTGC-3′ and 5′-GGTAAG-

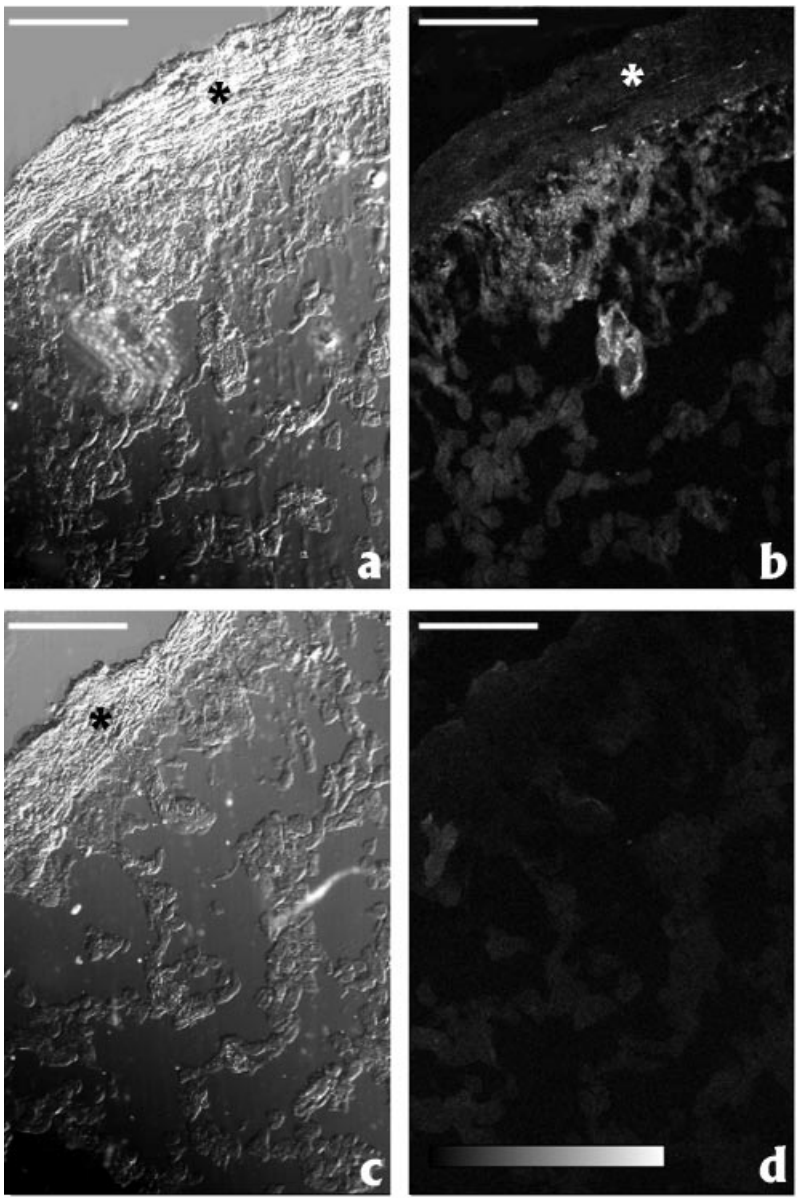

Fig. 1. Disposition of fluorescein isothiocyanate label following indirect immunofluorescence localisation of tPA in human late gestational placental basal plate region under the following conditions. (*a*) Nomarski differential interference microscopy showing the dry mass/refractive index properties of the section. The decidua (∗) is clearly distinct from the purely trophoblastic tissue. (*b*) Fluorescence microscopy showing distribution of tPA in the same section as *a*. Note absence of staining in the decidua basalis (∗). The trophoblast tissue of the cell columns and the extravillous trophoblast of the basal plate is immunoreactive. (*c*) Nomarski differential interference microscopy showing the dry mass/refractive index properties of this section. The decidua (∗) is clearly distinct from the purely trophoblastic tissue. (*d*) Same section as in *c* using immunofluorescence microscopy. Control experiment with omission of the specific antibody showing markedly reduced fluorescence intensity compared with *b*. Photographic exposure conditions and presentation as for *b*. Bars, 250 μm.

AAGTGTGAGACTCTCG3′; sequence (4) PAI-2: 5′-AACCAAAGGCAAAATCC-3′ and 5′-TACCTC-AACTTCATCTTCAGCC-3′.

These cDNA fragments were obtained by reverse transcription-PCR using total RNA from monkey placenta (Chomczynski & Sacchi, 1987). The tPA and PAI-1 cDNA fragments were ligated into pGEM-3Z vectors, and the uPA and PAI-2 cDNA fragments were ligated into pT7 vector and further subcloned into pGEM-3Z vectors. Restriction sites were unnecessary for ligation of sequences 3 and 4 into the vector as pT7 has 2 terminal thymidine bases at each end which bind to the adenine tails of the PCR product. Sequencing results revealed that monkey tPA. PAI-1, uPA and PAI-2 cDNA fragments have 95.78 %, 96.79 %, 95.90 % and 98.14 % identity with the corresponding human cDNA sequences respectively. Vectors were linearised such that antisense RNA probes could be obtained by use of SP6 RNA polymerases and the sense with T7. Dig-cRNA probes

were made using in vitro transcription kits (Promega) and RNA labelling mixture from Boehringer Mannheim.

In situ hybridisation

Procedures for in situ hybridisation were performed based on the method of Schaeren-Wiemer and Gerfin-Moser (1993) with digoxigenin-labelled monkey tPA, uPA, PAI-1 and PAI-2 riboprobes. Slides used for comparison were processed at the same time. To monitor background levels and the specificity of hybridisation, the sense strands of the corresponding monkey tPA, uPA, PAI-1 and PAI-2 were included in each experiment, respectively. Photographs were taken using E6 process colour film (Kodak and Fujichrome).

RESULTS

Since the distribution of protein and mRNA was similar in monkey and human placentae, where a single description is given it applies to tissues of both origins.

Cellular localisation of tPA protein and mRNA in human and rhesus monkey placentae

As shown in Figure 1, a strong tPA immunofluorescence was localised in human decidual cells at the level of stratum spongiosum of the decidua basalis. Cytotrophoblast cells of the basal and chorionic plates as well as along some areas in the septae also expressed tPA. Immunoreactivity to tPA was also found in the amnion membranes overlying human chorionic plate. No expression was observed in the basal plate endometrial stroma and chorionic plate connective tissue cells or in the septal stromal tissue.

On the whole, the distribution of tPA mRNA in human placenta is consistent with the tPA immunofluorescence localisation in the tissue (Fig. 2). There was relatively strong expression of tPA mRNA in cells with the morphological characteristics of extravillous trophoblast cells located in the basal plate (Fig. 2*a*). Positive expression of tPA mRNA was also noted in some isolated sites of trophoblast of chorionic villi (Fig. 2*c*), and in intima (Fig. 2*b*), particularly of fetal arterioles. There was no detectable expression in septal basal plate and basal plate endometrial stroma, fetal vessel media and adventitia, nor in chorionic plate connective tissue. The tPA sense strand negative control showed that these labelling patterns were specific (cf. Fig. 4*d*).

Expression of tPA mRNA in the monkey placenta was mainly concentrated in the trophoblast cells lining the chorionic and basal plate and septae. Positive anti tPa immunostaining of distal cytotrophoblast column cells at the points of attachment of anchoring villi was observed. Fairly extensive reaction product indicating tPa mRNA accumulated in villous trophoblast but not in the mesenchymal cores of villi. Nests of trophoblast nuclei were surrounded by cytoplasm with relatively strong reaction with the tPA mRNA probe.

Cellular localisation of uPA protein and mRNA in human and rhesus monkey placentae

Strong uPA immunoreactivity was observed in the cells of cytotrophoblast shell in the basal plate, possibly also in the mesenchyme and cytotrophoblast cells of villi, and in the decidual cells of the basal plate. No expression was detected in the basal and chorionic plate connective tissues and septal column tissue. In situ hybridisation analysis for uPA mRNA in human is shown in Figure 2*d*. The rhesus monkey placenta shows a similar distribution pattern. Strong expression was noted in the trophoblast cells overlying chorionic plate, basal plate and intercotyledonary septae. Expression was not observed in septal endometrial stromal tissues or regular connective tissue of basal plate region at detectably higher levels than in the sense strand control preparations (Fig. 4*d*).

Cellular localisation of PAI-1 protein and mRNA in human and rhesus monkey placentae

Confocal epifluorescence microscope analysis shows that anti-PAI-1 immunoreactivity was localised in cells of the basal plates of both human and rhesus monkey placentae. Sites of PAI-1 were observed in cells surrounding the blood vessel walls, extravillous trophoblast of basal plate and cytotrophoblast cells of the chorionic plate. Strong expression of PAI-1 immunoreactivity was noted in the decidual cells, along the edge of sites of separation from maternal tissues (Fig. 6).

In situ hybridisation of PAI-1 mRNA in the placentae of the 2 species further demonstrated that the extravillous trophoblast cells of the decidual layer between Rohr's and Nitabuch's striae (Fig. 3*b*) and

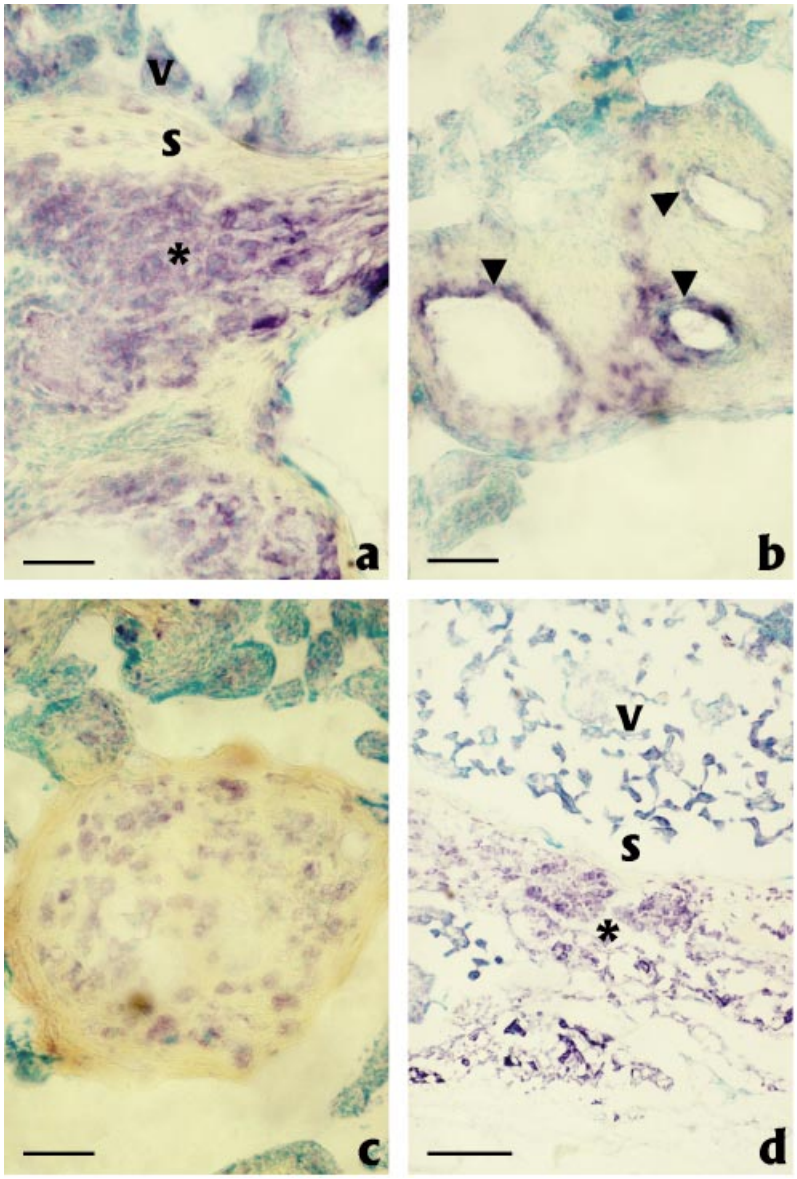

Fig. 2. All panels show in situ hybridisation of human late gestational placental tissue. The mRNA-containing cells are revealed by a strong purple enzyme histochemical reaction product. (*a*) Placental basal plate region. The villi (v) show weak tPA mRNA synthesis, the cells of the strial planes are negative (s) and some extravillous trophoblast cells show strong evidence of tPA mRNA production (*). Bar, 100 μm. (*b*) Transverse section through a stem villus. The endothelial cells of 3 blood vessels are sites of mRNA production for tPA (arrowheads). Bar, 100 μm. (*c*) Transverse section through fibrotic degenerate villus close to the chorionic plate. There is evidence of tPA mRNA in the core of this villus and in the mesenchymal cells of the cores of a few neighbouring villi. There is little/no evidence of synthesis in syncytiotrophoblast. Bar, 100 μm. (*d*) Placental basal plate region. The villi (v) stain green with the counterstain and show little evidence of uPA mRNA, the striae none (s) and the extravillous trophoblast cells of the basal plate region show considerable evidence of synthesis (*). Bar, 300 μm.

isolated or small groups of cytotrophoblast cells in the chorionic villi strongly expressed the messenger RNA for PAI-1. A low level of PAI-1 expression in the endothelial cells lining arterioles in stem villi (Fig. 6) was also observed. No expression was detected in chorionic plate, septae, villous core cells of mesenchymal type or in arteriolar media and adventitia.

The distribution of PAI-1 mRNA in monkey placenta was similar to that in human, but there was lower overall expression of this mRNA. Human cytotrophoblast underlying the chorionic plate in the nests of cells showed strong expression of PAI-1 mRNA. PAI-1 mRNA was also localised strongly in some large aggregates of basal plate extravillous trophoblast cells near to anchoring villi (Fig. 3*b*).

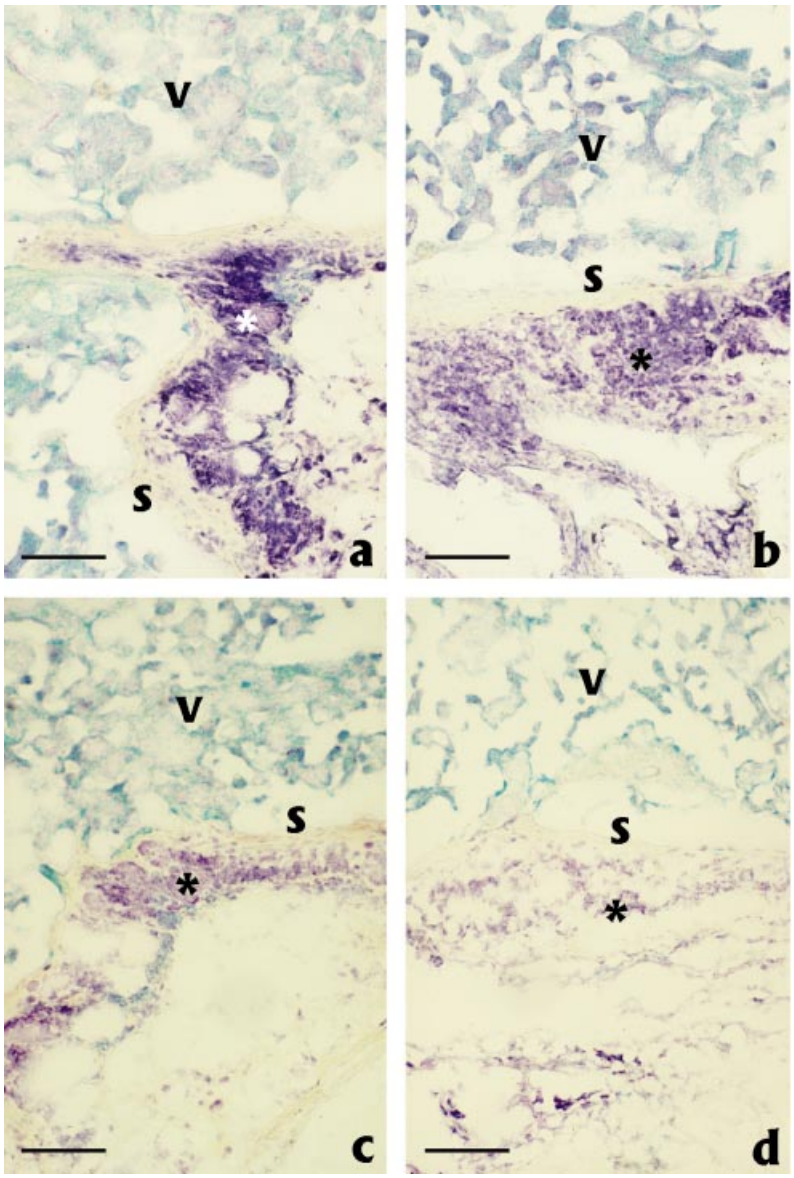

Fig. 3. All panels show aspects of human late gestational placental tissue. (*a*) Placental basal plate region. The villi (v) show little evidence of mRNA coding for PAI-1. The strial planes (s) are also negative but there is a strong signal showing mRNA for PAI-1 associated with extravillous trophoblast cells in the basal plate (*). Bar, 100 μm. (*b*) Placental basal plate region. The villi (v) show little evidence of mRNA coding for PAI-1. The strial planes (s) are also negative. There is evidence of relatively strong PAI-1 synthesis in an extensive population of basal plate extravillous trophoblast cells (*). Bar, 100 μm. (*c*) Placental basal plate region. The chorionic villi (v) show little evidence of mRNA coding for MMP, the strial planes are negative (s). There is evidence of a layer of extravillous trophoblast containing relatively large quantities of MMP mRNA (*). Bar, 100 μm. (*d*) Placental basal plate region. The villi (v) show little evidence of TIMP mRNA production, the strial plane none (s). There is evidence of a layer of extravillous trophoblast within the decidua showing relatively strong signal for TIMP mRNA (*). Bar, 100 μm.

Cellular expression of PAI-2 in human and rhesus monkey placenta

PAI-2 is a specific inhibitor for uPA, and thought to be of placental origin in normal pregnancy. Anti-PAI-2 immunofluorescence was found strongly concentrated in extravillous trophoblast cells of the basal plate and septae and in trophoblast of chorionic plate of both human and monkey placenta (Fig. 6). The immunoreaction was also evident in the trophoblast epithelium (Fig. 6). No detectable fluorescence was found in basal plate or septal endometrial stromal cells or the mesenchymal cores of the chorionic villi.

The distribution of PAI-2 mRNA is consistent with that of PAI-2 protein in the placentae. Expression of this messenger was strongest in extravillous cytotrophoblast cells of the basal plates and septae (Fig. 4*b*, *c*). Localisation of immunoreactivity of lower strength was observed in the trophoblast of chorionic villi (Fig. 4a–c). There was lack of detectable expression in the

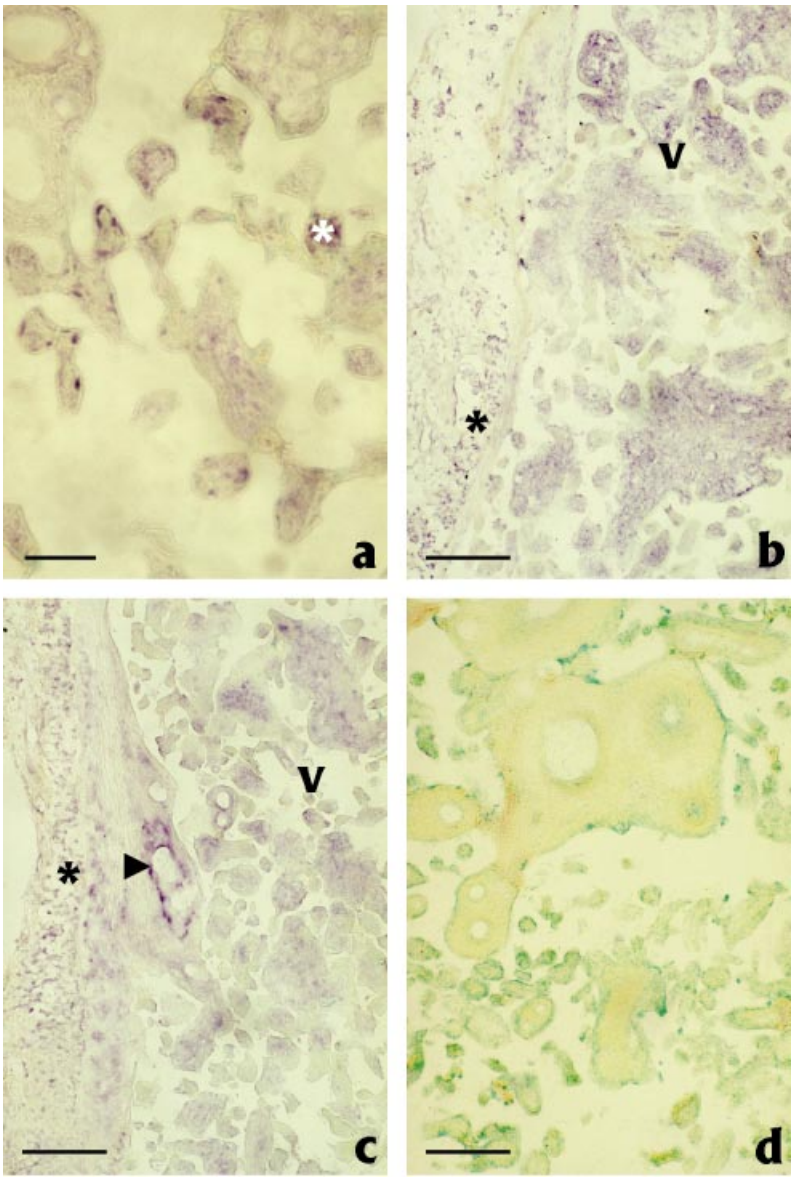

Fig. 4. All panels show aspects of human late gestational placental tissue. (*a*) Throughout the villi, widely separated irregular areas of reaction product deposition (see Fig. 6) are observed. This is evidence of locally restricted but relatively intense synthesis of mRNA for PAI-2 (*). Bar, 100 μm. (*b*) Placental basal plate region. The villi (v) show very little evidence of the presence of PAI-2 mRNA. There is slight evidence of the messenger in some extravillous trophoblast cells of the basal plate (*). Bar, 300 μm. (*c*) Placental basal plate region. The villi (v) show little evidence of PAI-2 mRNA. There is some evidence of the message within an anchoring villus (arrowhead) and in extravillous trophoblast of the basal plate (*). Bar, 300 μm. (*d*) An area of villous tissue representative of the appearance of all the sense-strand control experiments. Similar to this none of the probes gave positive signals in any areas examined. Bar, 300 μm.

mesenchymal layers of chorionic plate, septal endometrial stroma and mesenchymal core of chorionic villi of both human and monkey placentae.

Histological demonstration of fibrinoid

Using the triple stain MSB the overall distribution of fibrinoid in the tissue was revealed (Fig. 5*a–d*). There was fibrinoid related red-brown staining associated with fibrotic villi, associated with strial layers of the basal plate and in a thin layer associated with the chorionic plate trophoblast layer. These data indicate that the distribution of fibrinoid correlates with the overall distribution of cells containing and synthesising PAs and PAIs.

DISCUSSION

Local tissue breakdown in the placenta as a consequence of serine protease release under physiological control might be expected to satisfy at least 2

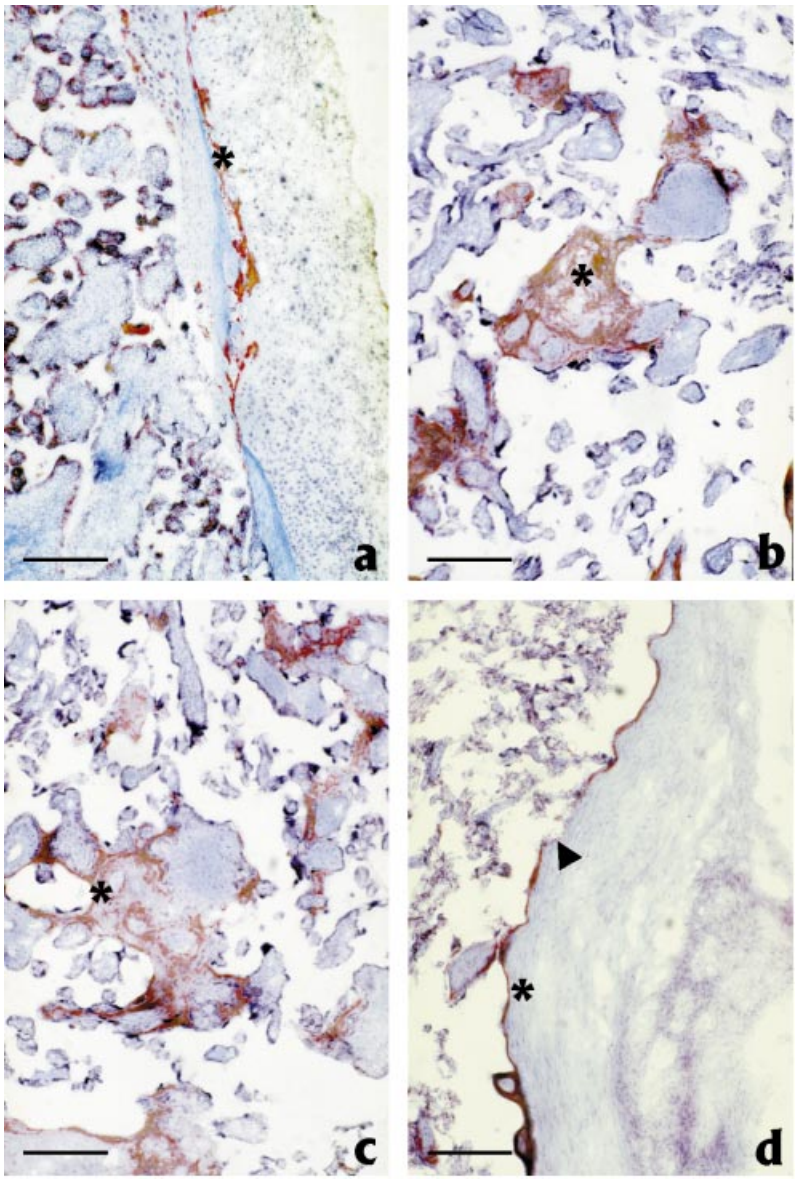

Fig. 5. Human late gestational placental tissue. MSB triple stain showing overall structure of the tissue and outlining the deposition of fibrinoid. In these preparations it stains a red-brown colour. (*a*) Basal plate regions showing Nitabuch's stria (*). Bar, 100 μm. (*b*) Fibrotic villus showing fibrin repair (*). Bar, 100 μm. (*c*) Fibrotic villi showing fibrin repair (*). Bar, 100 μm. (*d*) Chorionic plate showing the narrow layer of fibrin lining the intervillous space (arrowhead). Bar, 100 μm.

conditions. (1) That activators and inhibitors would colocalise but that the degree of expression would be temporally variable with one or other predominating at different gestational stages. (2) It would be more efficient and therefore probable that the site of synthesis of molecules affecting the rate of proteolysis would be localised close to the site of the preferred substrate for the proteolytic enzyme. The first of these conditions is clearly satisfied by our data which show considerable similarity in the distribution of the activators (tPA and uPA) and their inhibitors (PAI-1 and PAI-2) and of their respective messenger RNAs.

The plasminogen activators are believed to have a role in tissue breakdown as a consequence of their ability to generate plasmin. Whilst plasmin has a broad range of substrate specificities, encompassing many extracellular matrix molecules, the 'classical' substrate is fibrin (Vassalli et al. 1991).

It is clearly worth considering whether the pattern of distribution of the synthesis and expression of activators and inhibitors coincides with the distribution of fibrin-like molecules. The earliest studies of fibrinoid distribution in placental tissue are found in the classical literature of the 19th century and descriptions of Rohr's (1889), Nitabuch's (1887) and Langhans' striae, but an excellent review of the

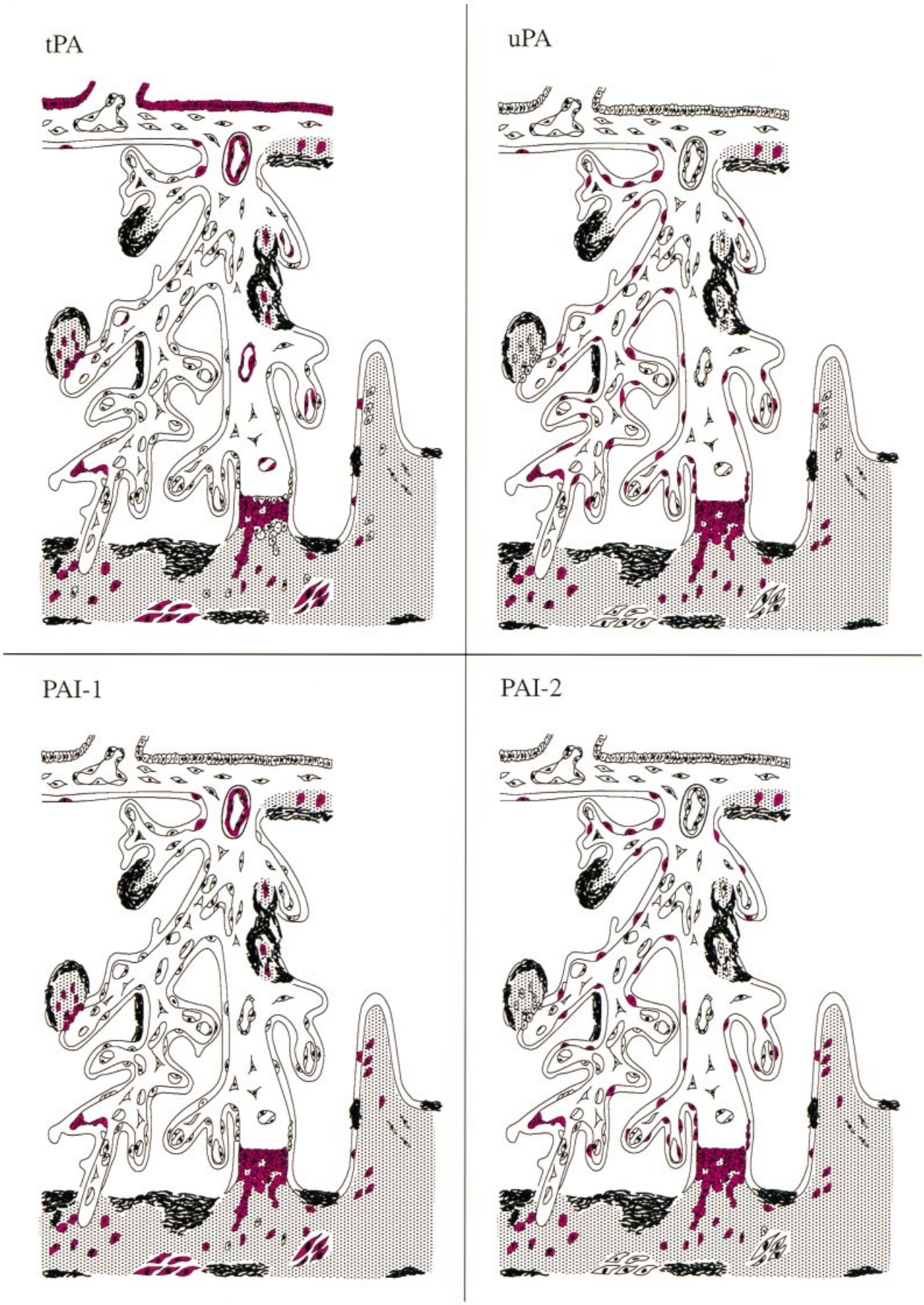

Fig. 6. For legend see opposite.

distribution of fibrinoid based on modern immunohistochemistry has described essentially 2 types (Frank et al. 1994) and defined the distribution of these. We include histological confirmation of some of these data in Figure 5. It is of interest that broadly the pattern of expression of activators and inhibitors can be interpreted as coinciding with the distribution of fibrinoid. Thus the second condition cited above is also satisfied at least in part.

The fibrinoid of the placenta and basal plate region is of 2 immunochemically distinguishable types: fibrin-type which contains fibrin and is essentially a repair protein which forms as a clot at surfaces wetted by blood, and matrix-type which contains the 'tissue glue' oncofetal fibronectin (Feinberg et al. 1991; Frank et al. 1994). Both types of fibrinoid may be affected directly or indirectly by the plasminogen activators or their inhibitors. Indeed, according to many observers, both types of fibrinoid coexist and matrix-type fibrinoid molecules have the power to initiate clotting thus accounting for the frequently observed superficial coating of matrix-type fibrinoid with fibrin-type fibrinoid (Coleman et al. 1987).

The matrix-type fibrinoid is expected to be an important element in implantation and in the abscission process during the third stage of labour. The secretion of oncofetal fibronectin is a consequence of activity by fetal epithelial (trophoblast) cells. Within the basal plate and the chorion layer these cytotrophoblast cells secrete large quantities of extracellular matrix in a nonpolar fashion. The secretions combine collagen IV, laminin, and cellular fibronectin, oncofetal fibronectin and heparan sulphate glycoprotein (Castellucci et al. 1993; Nanaev et al. 1993). These cells also figure prominently in the expression patterns of antigen and messenger RNA for the activators and inhibitors along with the decidual cells that lie so very close to them. One can perhaps predict from these observations that the autocrine or paracrine controls over this system work at rather short ranges of the order of 100 μm.

In this way it is possible that a *narrow* band of relatively intense serine protease activity might result in a neat abscission process while preserving the majority of the tissue of the uterine wall, fetus, placenta and membranes. It would be of interest to examine the expression of the probes used here on sections derived from Caesarian delivered placentae at term and from Caesarian preterm deliveries in order to establish any differences that may exist related specifically to parturition.

The local failure of the degradative processes at term may cause retention of placental products which are associated with risks of post-partum haemorrhage. The lack of synthesis of inhibitors or excessive synthesis of activators may be responsible for cases of placental abruption prior to term and retroplacental haematoma.

During implantation the ratio of matrix to fibrin-type fibrinoid may be important in attachment, nidation (interstitial implantation) and maternal vascular invasion leading to the establishment of effective haematotrophic nutrition at least by the end of the first trimester (Frank et al. 1994) and to the control of these processes. The normal barrier properties of fibrin-type fibrinoid may be overcome by the plasminogen activators dominating their inhibitors and leading to pathologically invasive forms of placentation such as placenta accreta, placenta increta and placenta percreta. In these conditions the relatively poor blood supply to the cervix may limit the supply of fibrin, exacerbating the problem and leading to the local predominance of the condition.

In summary, it is possible that the activators and inhibitors described here interact at a variety of crucial physiological stages of pregnancy. They appear to do this in tightly restricted locations and at specific times, particularly early and late in pregnancy, to promote healthy gestation. It is possible that failure of the local controls over the system are important in a range of gestational pathologies. These include failure of implantation and premature rupture of membranes as others have noted (Liu et al. 1998). However, we now propose that the location of the production of these molecules gives the system potential in the control over abscission during the third stage of labour and predict a role in the pathology of abruptio placenta and retained placental products. Other

Fig. 6. Diagram summarising the conclusions regarding the dispositions of the activators and inhibitors of plasminogen and their sites of synthesis. These positions are denoted in one set of diagrams as the locations of each protein and its mRNA are coincident in both human and simian placentae at late preterm stages of gestation (see Materials and Methods). The base diagram used is redrawn and modified from the original work of Frank et al. (1994) describing the location of fibrinoids of 2 types in the human placenta (matrix-type fibrinoid: stipple, fibrin-type fibrinoid: black fibres). The disposition of fibrin (the major substrate for plasmin) is predictable in several locations (e.g. Nitabuch's stria) whereas its association with restricted regions of repair to the damaged parts of the chorionic villus tree is rather variable. It is possible that foci of activator and inhibitor synthesis within villus tree trophoblast are coincident with these foci, but formal evidence of this awaits triple labelling studies: in situ hybridisation of appropriate activator and inhibitor mRNAs and antifibrin immunohistochemistry. The association with the regions which predictably contain fibrin is supported by the present data.

significant pathological associations may include placenta accreta, increta and percreta.

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