

Alterations in distribution and composition of the extracellular matrix during decidualization of the human endometrium

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The distribution of the extracellular matrix, including type I, III, IV and VI collagens and laminin, and of prolyl hydroxylase was investigated in the human endometrium by an indirect immunofluorescence method with specific monoclonal antibodies. Collagens were also extracted from the endometrial tissues in the proliferative and secretory phases and from the decidual tissues in the first trimester of pregnancy. Immunohistochemical studies demonstrated that interstitial collagens, such as type I, III, and VI collagens, were localized diffusely in the stroma of the endometrium throughout the menstrual cycle, as well as in the decidua. Type IV collagen and laminin were localized exclusively in the basement membrane of the endometrial glands and in the walls of blood vessels during the proliferative and secretory phases. However, strong staining for type IV collagen and laminin was recognized in the pericellular region of endometrial stromal cells in the decidua. Prolyl hydroxylase was localized in the cytoplasm of endometrial stromal cells and endometrial glandular cells during the menstrual cycle. Intense immunostaining for prolyl hydroxylase was observed in the decidual cells. However, immunoreactivity for prolyl hydroxylase in the endometrial glandular cells disappeared during the process of decidualization. The ratio of type III to type I collagen was significantly decreased ($P < 0.05$) and the ratio of type V to type I collagen was significantly increased ($P < 0.01$) in the decidua, as compared with ratios in the endometrium during the proliferative phase. The present results suggest that changes in the extracellular matrix may play an important role in implantation, in invasion of trophoblastic cells and in the maintenance of pregnancy.

Introduction

The extracellular matrix (ECM) is considered to play an important role in the stability of tissue structure and in the regulation of cell growth and differentiation (Labat-Robert *et al.*, 1990; Madri and Basson, 1992; Lin and Bissell, 1993). Indeed, the synthesis, accumulation and catabolism of ECM are involved in wound healing and in the initiation and progression of several diseases, such as atherosclerosis, liver fibrosis, glomerulosclerosis, glomerulonephritis, scleroderma, and pulmonary fibrosis (Haralson, 1993).

During the menstrual cycle and early pregnancy, pronounced morphological changes occur in the human endometrium under the control of ovarian hormones, which include oestrogen and progesterin (Strauss and Gurpide, 1991). Endometrial tissues are considered to play a role in implantation of the embryo, control of trophoblast invasion (Pijnenborg *et al.*, 1980), nutrition of the blastocyst (Kearns and Lala, 1983), secretion of prolactin (Maslar *et al.*, 1980; Daly *et al.*, 1983), protection of the embryo from the maternal immune system

(Golander *et al.*, 1981), and the synthesis of ECM (Juan *et al.*, 1989). Appropriate changes in the quantity and composition of ECM, as well as the relationship between the ECM and endometrial cells, must surely be essential for implantation of the embryo, invasion of trophoblastic cells into the decidualized endometrium, and the maintenance of pregnancy. Collagens and laminin are major components of the ECM. Research into collagen in the past decade has resulted in the identification of at least 19 different types of collagen plus numerous other nonstructural proteins that contain at least one collagen triple helix as a structural motif (Van der Rest and Garrone, 1991); all of which are regarded as members of a collagen superfamily (Hulmes, 1992). Interstitial and basement membrane collagens, glycoproteins, and proteoglycans in the endometrium have been reported to change markedly with changes in hormone concentrations (Aplin *et al.*, 1988). However, little is known about alterations in the distribution and composition of the ECM in the human endometrium and decidua. In the present study, therefore, we investigated changes in the distribution of the ECM by an indirect immunofluorescence method using specific antibodies directed against the various components of the ECM, including type I, III, IV, and VI collagens and laminin, and using a specific antibody

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against prolyl hydroxylase, which is a key enzyme in the synthesis of collagen (Rhoads and Udenfriend, 1970; Cardinale and Udenfriend, 1974). We also discuss the relative amounts of various collagens in the endometrial tissues in the proliferative and secretory phases and in the decidual tissues during the first trimester of pregnancy.

Type V collagen is a very poor immunogen against which it has been difficult to prepare reliable specific antibodies. Therefore, we evaluated the change in the relative amount of this collagen in the endometrium from the proliferative phase to early pregnancy.

Materials and Methods

Tissues

Human endometrial tissues were obtained by endometrial biopsy and hysterectomy for gynaecologic indications, such as uterine myoma, and were immediately frozen in liquid nitrogen. Decidual tissues were obtained by dilatation and curettage for termination of pregnancy. The project was approved by the Committee on Investigations Involving Human Subjects of Wakayama Medical College. Informed consent was obtained from each woman after the purpose and nature of the study had been fully explained. The endometrial phase was determined by the timing of the last menstrual period, the records of basal body temperature, and the histology. We excluded endometrial tissues in which obvious inflammation was present. Thirty-six samples of proliferative-phase endometrium, 42 samples of secretory-phase endometrium, and 53 samples of decidual tissues from early pregnancies (7 to 12 weeks) were examined. Gestational age was determined from the date of the last menstrual period and ultrasonographic measurements during early pregnancy.

Primary antibodies

Monoclonal antibodies (mAbs) against human type I, III, IV and VI collagens were used for this study. Preparation of the antibodies has been described by Ooshima and Muragaki (1990). In brief, BALB/C mice were immunized with each collagen, which had been extracted from human placentas. The spleen cells were hybridized with myeloma cells. After HAT (hypoxanthine-aminopterin-thymidine) selection, positive hybrids were screened by enzyme-linked immunosorbent assay. The specificity of each antibody was determined by an immunoblot or inhibition enzyme-linked immunosorbent assay. No crossreaction was recognized among the antibodies against each human collagen. Polyclonal antibodies against human laminin were purchased from Gibco Lab. Co. (Grand Island, NY). The laminin-specific antibody also showed no crossreaction with type I, III, IV, and VI collagens or fibronectin. Monoclonal antibodies against prolyl hydroxylase (anti-HPH(β); horseradish peroxidase-conjugated Fab' (Fab'-HRP)), which recognized the β subunit of prolyl 4-hydroxylase, were prepared as described by Bai *et al.* (1986).

Immunohistochemistry

Immunohistochemistry was performed by the indirect immunofluorescence method. In brief, 3 μ m frozen sections

were rehydrated in PBS at room temperature and then incubated with the primary antibody (1:100 diluted in PBS) at 4 °C for 12 h in a humidified chamber. After incubation, the sections were washed twice in PBS for 3 min. Each slide was then incubated at room temperature for 1 h with human plasma-preabsorbed, fluorescein isothiocyanate-conjugated goat antibodies against mouse immunoglobulins or goat antibodies against rabbit immunoglobulins that had been diluted 1:100 (Organon Teknik, Co., West Chester, PA). The sections were washed again in PBS, mounted in buffered glycerol and examined under a fluorescence microscope (Olympus Co., Tokyo). Control sections were stained with goat antibodies against mouse immunoglobulin G (secondary antibodies) without prior application of the appropriate primary antibody. When the mAbs were first absorbed with an excess of each type of collagen, laminin or prolyl hydroxylase, as appropriate, no immunostaining was observed (data not shown).

SDS-PAGE of pepsin-solubilized collagens from the human endometrium

Minced samples of human endometrium were washed overnight in cold distilled water and freed of blood. Tissues were homogenized with a Polytron homogenizer in 50 volumes of 0.5 mol acetic acid l^{-1} that contained 1 mg pepsin ml^{-1} (Sigma Chemical Co. St Louis, MO). Collagens were extracted with constant stirring for 24 h at 4°C. The solutions were centrifuged at 39 000 g for 1 h at 4°C. Collagens were re-extracted from the pellets under the same conditions for 48 h. The supernatants were then combined and collagens were precipitated by addition of 4.0 mol NaCl l^{-1} to a final concentration of 2.0 mol l^{-1} . The precipitate was dissolved in 0.5 mol acetic acid l^{-1} and dialysed against 0.02 mol Na_2HPO_4 l^{-1} . Precipitated collagens were redissolved in 0.5 mol acetic acid l^{-1} , dialysed exhaustively against 0.05 mol acetic acid l^{-1} and finally lyophilized. The solubility of the tissue collagen from each endometrial sample was estimated by comparing the hydroxyproline content of the initial homogenate with that of the final solution of collagen (Kivirikko and Prockop, 1967). Type V collagen was isolated by salt precipitation from pepsin digests of human decidual tissues by the methods described elsewhere (Furuto and Miller, 1980; Miller and Rhodes, 1982). The extracted type V collagen was also lyophilized. Estimation of the relative abundance of the $\alpha 1(III)$ chain and the $\alpha 1(V)$ chain was performed by interrupted gel electrophoresis (Sykes *et al.*, 1976). Electrophoresis was performed in an 8% polyacrylamide gel slab (Sigma Chemical Co.). The gel and electrode buffers were 0.1 mol phosphate buffer l^{-1} , pH 7.2, containing 0.1% (w/v) SDS (Nacalai Tesque Inc., Kyoto), as described by Laemmli (1970). Lyophilized samples of collagens and type V collagen were dissolved at a concentration of 0.2 mg ml^{-1} and denatured by heating in the gel buffer that contained 1% (w/v) SDS at 60°C for 30 min.

Aliquots of 25 μ l of solutions of denatured collagens and 5 μ l of denatured type V collagen were applied to the gel and subjected to electrophoresis at 80 mA. After 1.5 h the current was switched off and sample wells were filled with 20% (w/v) β -mercaptoethanol (Wako Chemical Co., Osaka), which was allowed to diffuse into the gel for 1 h to cleave intramolecular

disulfide bonds of type III collagen [$\alpha 1(\text{III})_3$]. Then electrophoresis was resumed and allowed to continue for 1 h. Each collagen α chain was stained with Coomassie brilliant blue (Sigma Chemical Co.) and quantitated by densitometry. The relative amounts of $\alpha 1(\text{III})$ or $\alpha 1(\text{V})$ chains were calculated by dividing the intensities of bands of $\alpha 1(\text{III})$ and $\alpha 1(\text{V})$ by that of $\alpha 1(\text{I})$.

Statistical analysis

The ratio of $\alpha 1(\text{III})$ to $\alpha 1(\text{I})$ chains and that of $\alpha 1(\text{V})$ to $\alpha 1(\text{I})$ chains, as estimated by densitometry, were represented as means \pm SEM, and they were analysed by analysis of variance and Student's *t* test.

Results

Immunohistochemical analysis of the endometrium and decidua

Enhanced immunostaining with the mAbs against type I (Fig. 1a, b) and type III (Fig. 1d, e) collagens was observed with a fibrillar pattern in the stroma of the endometrium and in the vessel walls throughout the menstrual cycle. Immunostaining with the mAb against type VI collagen was also recognized in the stroma of the endometrium during the menstrual cycle, with a fine fibrillar pattern (Fig. 1g, h). In the decidual tissue, there was strong immunostaining with mAbs against type I, III, and VI collagens in the matrix between decidual cells and in the vessel walls (Fig. 1c, f, i). By contrast, no immunostaining for type I, III, and VI collagens was observed in endometrial glands.

Immunostaining with the mAb against type IV collagen was observed exclusively on the basement membrane of endometrial glands and beneath the endothelial cells of capillaries throughout the menstrual cycle (Fig. 2a, b). In decidual tissues, by contrast, type IV collagen was observed in the pericellular region around the decidual cells with strong immunofluorescence (Fig. 2c, g). Immunoreactive staining with the specific antibodies against laminin was observed on the basement membrane of the endometrial glands and beneath the endothelial cells of capillaries (Fig. 2d, e). Strong immunofluorescence specific for laminin was observed in the pericellular aura of the decidual cells with a similar pattern of staining to that of type IV collagen (Fig. 2f, h).

Throughout the menstrual cycle, the stromal cells of the endometrium exhibited positive immunoreactivity for prolyl hydroxylase as a fine granular pattern in the cytoplasm (Fig. 3a, b). The epithelial cells of the endometrial glands (Fig. 3a, b) and the endothelial cells of vessels (Fig. 3c) were also immunopositive for prolyl hydroxylase. Moreover, enhanced immunoreactivity specific for prolyl hydroxylase in the cytoplasm of the decidual cells gave a fine granular pattern (Fig. 3c). In the decidua, however, only weak immunofluorescence in the epithelial cells of endometrial glands was detected (Fig. 3d).

Interrupted SDS-PAGE of pepsin-solubilized collagens from the endometrium and decidua

The interrupted SDS-PAGE revealed that the relative amounts of $\alpha 1(\text{V})$ and $\alpha 1(\text{I})$ increased and that of $\alpha 1(\text{III})$

decreased from the proliferative-phase endometrium to the decidua (Fig. 4). The ratios of intensities of bands of $\alpha 1(\text{III})$ to $\alpha 1(\text{I})$ (Fig. 5a) and $\alpha 1(\text{V})$ to $\alpha 1(\text{I})$ (Fig. 5b) were 0.76 ± 0.03 , 0.47 ± 0.09 , 0.37 ± 0.04 , and 0.033 ± 0.014 , 0.057 ± 0.015 , 0.107 ± 0.010 (mean \pm SEM) in the proliferative-phase endometrium, the secretory-phase endometrium, and in the decidua, respectively. The ratios of intensities of bands of $\alpha 1(\text{III})$ to $\alpha 1(\text{I})$ in the secretory-phase endometrium and the decidua were significantly lower than that in the proliferative-phase endometrium ($P < 0.05$). Furthermore, the ratio of intensities of $\alpha 1(\text{V})$ to $\alpha 1(\text{I})$ in the decidua was significantly higher than that in the proliferative-phase endometrium ($P < 0.01$) and than that in the secretory-phase endometrium ($P < 0.05$).

Discussion

In the present study, we investigated changes in the distribution and composition of the ECM, including type I, III, IV, V and VI collagens and laminin, as well as in the distribution of prolyl hydroxylase, in the human endometrium throughout the menstrual cycle and in the decidua during early pregnancy. We were able to solubilize 70–85% of collagen in the human endometrial and decidual tissues, as measured by reference to amounts of hydroxyproline present (data not shown). Therefore, it was postulated that the extracted collagen accurately reflects the entire complement of collagen in the tissues.

The presence of interstitial collagens, such as type I, III, and VI collagens, was confirmed immunohistochemically in the endometrium throughout the menstrual cycle and in the decidua. Type I and type III collagens were distributed in a diffuse and fibrillar pattern in both the proliferative-phase and secretory-phase endometrium and were more tightly packed between the decidual cells. Although type I and type III collagens are commonly found in combination, the ratio of type III to type I collagen in the proliferative-phase endometrium was significantly higher than the ratios in the secretory-phase endometrium and decidua. Changes in the ratio of type III to type I collagen have been demonstrated in human skin (Sykes *et al.*, 1976) and in human atherosclerosis (McCullagh and Balian, 1975; Ooshima, 1981). A possible cause of the change in the composition of the ECM may be an alteration in the hormonal environment, which includes oestrogen and progesterin. Another cause may be an alteration in the density of cells in the human endometrium. Cell density-dependent effects have been reported in the various types of cell, such as mesangial cells (Ishimura *et al.*, 1989; Lermioglu *et al.*, 1991; Worthuis *et al.*, 1993), endothelial cells (Patton *et al.*, 1990), vascular smooth muscle cells (Campbell *et al.*, 1989; Goodman and Majack, 1989; Majors and Ehrhart, 1992), fibroblasts (Halme *et al.*, 1986; Rösner *et al.*, 1990), and primitive mesenchymal cells (Tsonis and Goetinck, 1990). It has been suggested that cell density modulates biological behaviour, with changes in signal-transduction responses to hormonal stimulation, in growth, in the synthesis and composition of the ECM, and in the synthesis of specific proteins (Ishimura *et al.*, 1989; Lermioglu *et al.*, 1991; Worthuis *et al.*, 1993). Worthuis *et al.* (1993) reported that mesangial cells synthesized relatively more type I collagen per cell at higher cell densities, whereas synthesis of type III and type IV

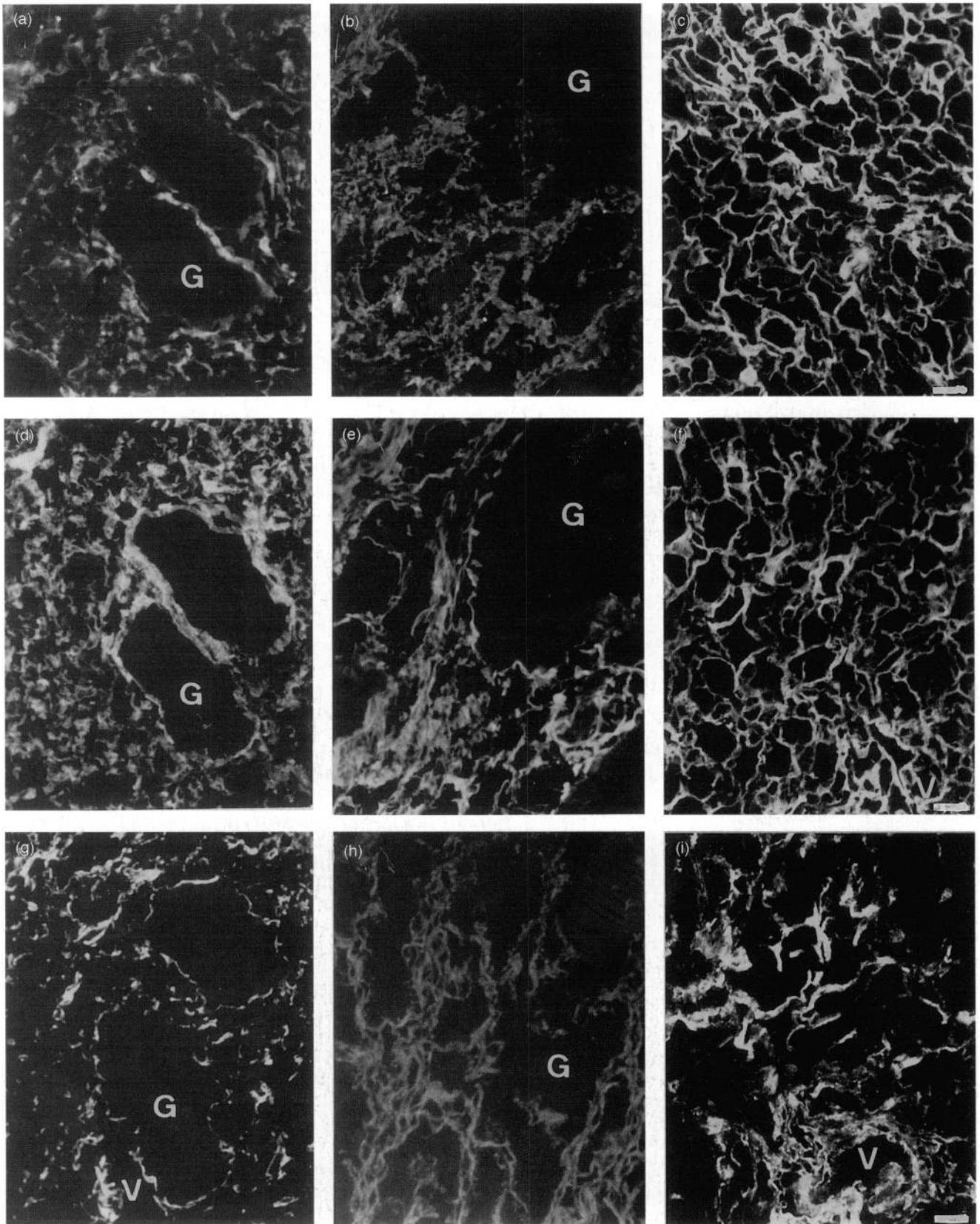


Fig. 1. Immunofluorescence micrographs of human endometrium and decidua with monoclonal antibodies specific for collagen type I (a–c), III (d–f), and VI (g–i): proliferative phase endometrium: a, d, g; secretory-phase endometrium: b, e, h; decidua: c, f, i. Strong immunofluorescence specific for each collagen was distributed diffusely in the stroma of endometrial and decidua tissues. Immunostaining for each collagen was visible in blood vessels (V) but not in endometrial glands (G). Scale bars represent 50 μ m.

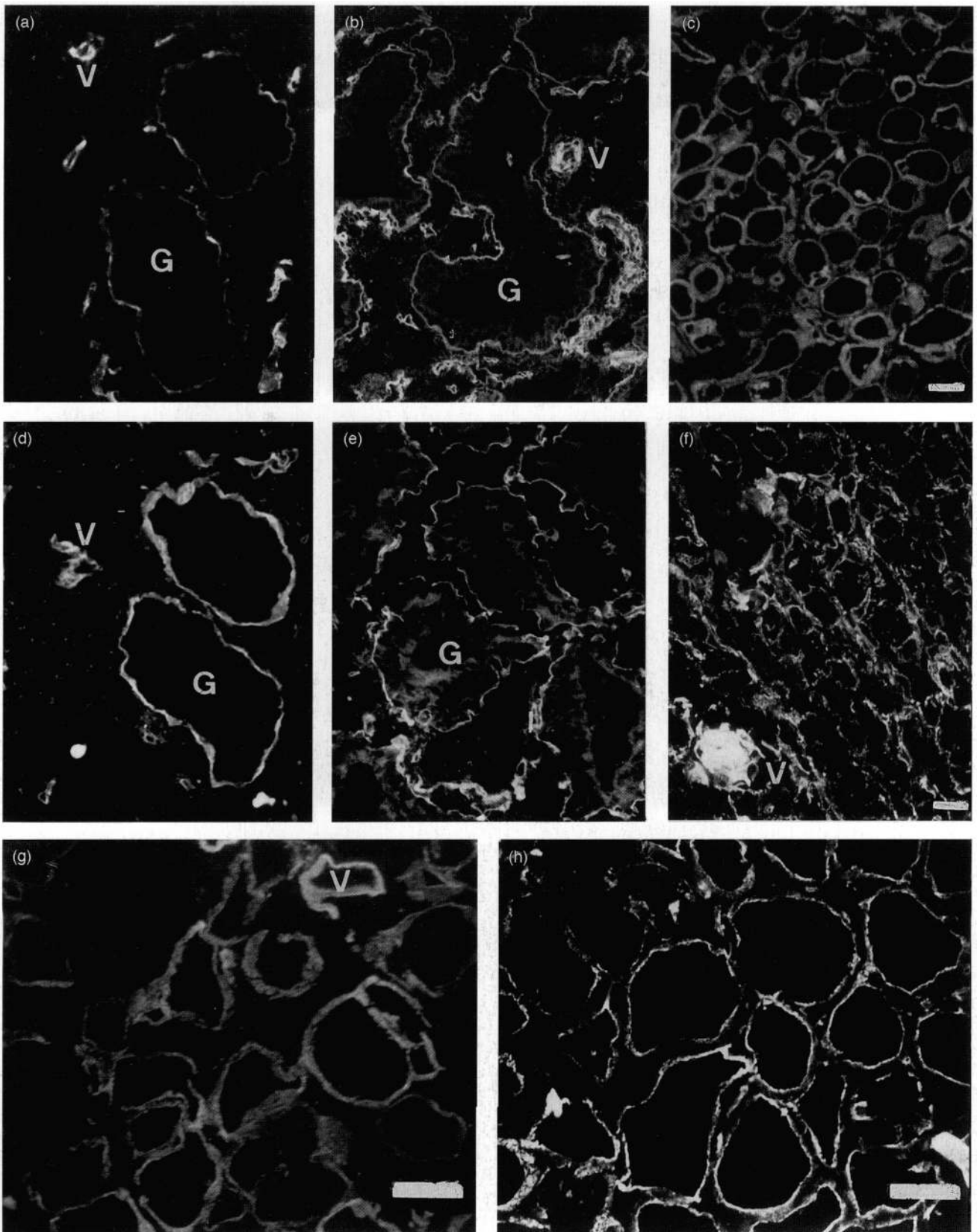


Fig. 2. Immunofluorescence micrographs of human endometrium and decidua stained with a monoclonal antibody specific for collagen type IV (a–c, g) and polyclonal antibodies against laminin (d–f, h) (proliferative-phase endometrium: a, d; secretory-phase endometrium: b, e; decidua: c, f, g, h). In the proliferative-phase and secretory-phase endometrium, type IV collagen and laminin were localized exclusively in the basement membranes of endometrial glands (G) and blood vessels (V). In the decidua, intense immunostaining for type IV collagen and laminin was recognized in the pericellular region of decidual cells. Scale bars represent 50 μ m.

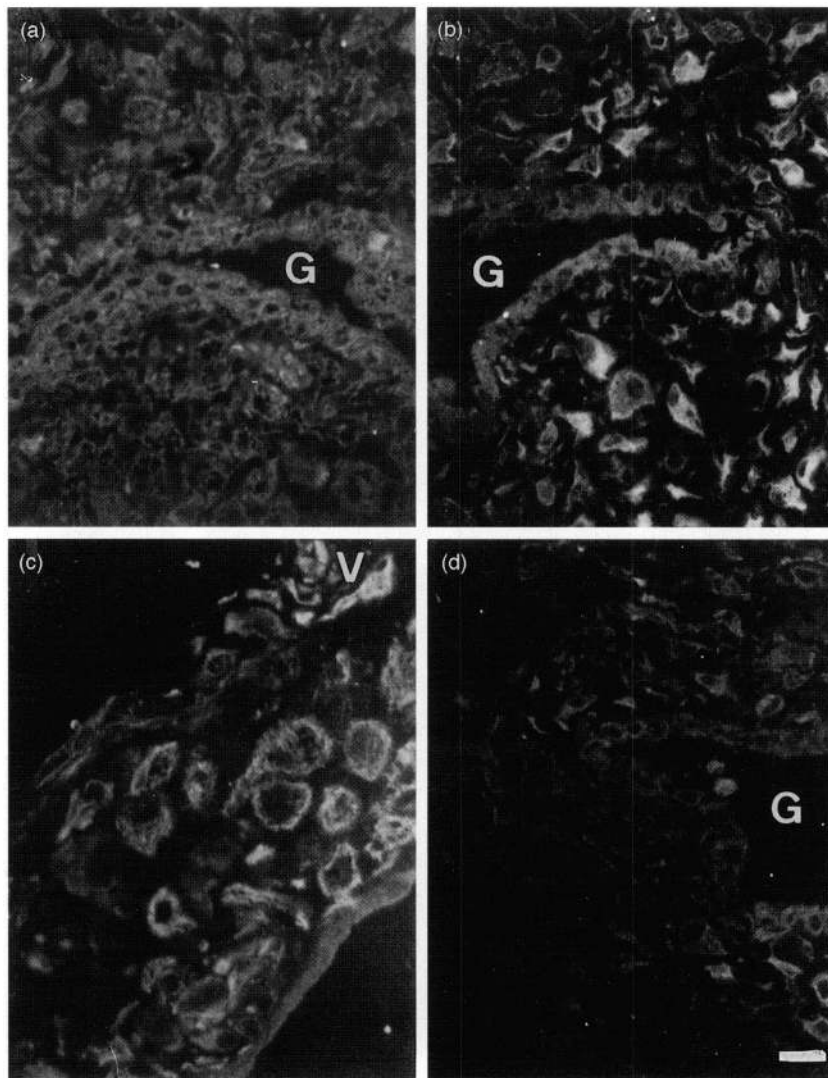


Fig. 3. Immunofluorescence staining for human prolyl hydroxylase in the secretory-phase endometrium and decidual tissue (proliferative-phase endometrium: a; secretory-phase endometrium: b; decidual tissue: c, d). Immunostaining was observed in the endometrial stromal and glandular cells in the proliferative and secretory-phase endometrium (a, b). Decidual cells showed enhanced immunostaining for prolyl hydroxylase (c). Endometrial glandular cells showed very weak or no immunofluorescence in the decidua (d). G: endometrial gland; V: blood vessels. Scale bar represents 50 μ m.

collagens in each cell did not depend on cell density. Therefore, it is suggested that ovarian secretion of oestrogen causes intense proliferation of the endometrial stromal cells during the proliferative phase and that, at higher cell densities, stromal cells may synthesize specifically type I collagen predominantly during the secretory phase and during the first trimester of pregnancy, as a result of prolonged exposure to progesterin. In the present study with a mAb against type VI collagen, type VI collagen was found to be localized diffusely in the human endometrium in a fine fibrillary pattern throughout the menstrual cycle and in the decidua in the first trimester. These findings are not consistent with the previously published data (Aplin *et al.*, 1988; Mulholland *et al.*, 1992). This microfibrillar collagen is an unusual and unique collagen, which may function as an anchor to the surrounding ECM (Bruns *et al.*, 1986; Keene

et al., 1988). Mulholland *et al.* (1992) indicated that type VI collagen is lost from the rat endometrial stroma during decidualization, whereas this collagen begins to reappear in the ECM after the onset of regression of decidual cells, suggesting that type VI collagen plays a role in trophoblastic invasion. The results of our observations suggest the reappearance of type VI collagen in the human decidual tissues. In the decidua, components of the basement membrane, such as type IV collagen and laminin, were apparently present around the decidual cells. This finding is in agreement with previous reports (Ulla *et al.*, 1985; Aplin *et al.*, 1988). The accumulation of pericellular, basement membrane-like substances, including type IV collagen and laminin, may be associated with the process of decidualization of the endometrium. The basement membrane may be involved in selective permeability of macromolecules and structural

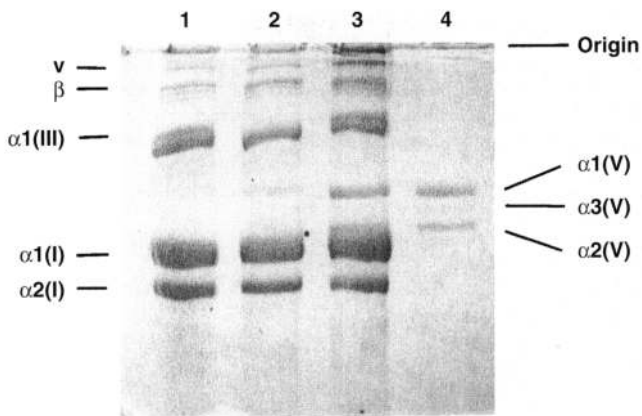


Fig. 4. SDS-PAGE of pepsin-solubilized human collagens from the proliferative-phase endometrium (lane 1), secretory-phase endometrium (lane 2), decidua (lane 3), and type V collagen extracted from the decidual tissues (lane 4). Samples of heat-denatured collagen (5 μ g, lanes 1–3; 1.0 μ g, lane 4) were subjected to electrophoresis on a slab gel for 1 h. After reduction *in situ* with β -mercaptoethanol for 30 min, electrophoresis was resumed for 1 h. In this study, the α chains derived from type IV collagen could not be detected because of their low concentration in the sample. Note that the type V collagen obtained from decidual tissue by differential salt precipitation was composed predominantly of the $\alpha 1(V)$ chain.

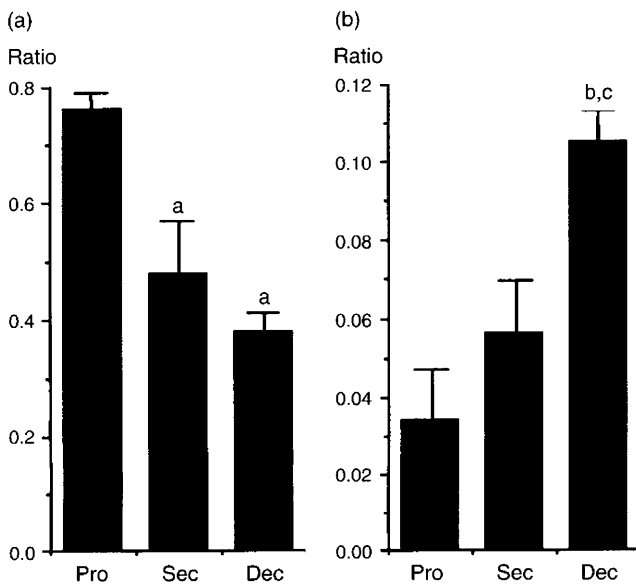


Fig. 5. Relative abundance of (a) the $\alpha 1(III)$ and (b) the $\alpha 1(V)$ chains of human collagen as compared with the $\alpha 1(I)$ chain in the proliferative-phase endometrium (Pro; $n = 36$), the secretory-phase endometrium (Sec; $n = 42$), and the decidua (Dec; $n = 53$). Data are presented as the mean \pm SEM. Values were analysed for the statistical significance of differences among the three groups by analysis of variance and Student's *t* test. ^a $P < 0.05$ versus the proliferative-phase endometrium, ^b $P < 0.01$ versus the proliferative-phase endometrium, ^c $P < 0.05$ versus the secretory-phase endometrium.

tissue-support. In contrast, laminin, which binds to itself, to type IV collagen, to heparin, and to cell-surface receptors, promotes the adhesion and growth of various epithelial and tumour cells (Kleinman *et al.*, 1985; Martin and Timpl, 1987), as

well as the outgrowth of neurites (Baron van Evercooren *et al.*, 1982). In addition, recent studies have revealed that the amino acid sequence of laminin exhibits considerable homology to that of epidermal growth factor-related (EGF-related) proteins (Gray *et al.*, 1983; Sasaki *et al.*, 1987, 1988) and that both EGF and laminin stimulate cell growth and differentiation (Carpenter and Cohen, 1979). Thomas and Dziadek (1993) demonstrated that the gene for laminin is expressed in the decidua during the peri-implantation period. These findings suggest that pericellular accumulated laminin modulates the functions of decidual cells in an autocrine or paracrine fashion.

In the present study, we investigated the immunolocalization of prolyl hydroxylase in the proliferative and secretory endometrium and in the decidua. Immunostaining for prolyl hydroxylase was clearly apparent in the cytoplasm of stromal cells, epithelial cells of endometrial glands, and endothelial cells of vessels. These findings suggest that stromal cells are mainly responsible for the synthesis of collagen in the endometrium. In the decidua, strong immunoreactivity was observed in the cytoplasm of the decidual cells, suggesting that decidual cells synthesize various types of collagen more actively than stromal cells in the proliferative and secretory endometrium. Prolyl hydroxylase was also localized in the epithelial cells of endometrial glands and, therefore, endometrial glandular cells may be involved in the synthesis of basement membrane collagen during the proliferation of endometrial glands. In the decidua, the disappearance of immunoreactivity for prolyl hydroxylase in the endometrial glandular cells may reflect the regression of endometrial glands. Prolyl hydroxylase, a key enzyme in the hydroxylation of proline to hydroxyproline during the synthesis of collagen (Rhoads and Udenfriend, 1970; Cardinale and Udenfriend, 1974), is a tetramer of two α and two β subunits (Kivirikko *et al.*, 1989). A cDNA sequence for the β subunit of human prolyl hydroxylase has been found to be highly homologous with that for a rat protein disulfide isomerase, which is regarded as the catalyst *in vivo* for formation of disulfide bonds in the biosynthesis of various secretory proteins (Edman *et al.*, 1989; Kivirikko *et al.*, 1989). Therefore, the immunoreactivity of the β subunit may reflect the enzymatic activity of prolyl hydroxylase in collagen metabolism.

In the case of type V collagen, the purified protein extracted from the decidua by differential salt precipitation was composed of $\alpha 1(V)$, $\alpha 2(V)$ and $\alpha 3(V)$ chains, and $\alpha 1(V)$ was the predominant chain. The present study showed that the $\alpha 2(V)$ chain comigrated with the $\alpha 1(I)$ chain, and the $\alpha 3(V)$ chain was almost undetectable after SDS-PAGE. Therefore, the ratios of $\alpha 1(III)$ and $\alpha 1(V)$ calculated in terms of $\alpha 1(I)$ are likely to be something of an underestimate. Nevertheless, the marked increase in the intensity of the band of $\alpha 1(V)$ was quite evident in the case of decidua. The most striking finding was a significant increase in the relative amount of type V collagen in the decidua as compared with that in the proliferative-phase and secretory-phase endometrium. Thus, it may be concluded that decidual cells, which are the major collagen-producing cells, synthesize predominantly type V and type I collagens and lower amounts of type III collagen. Increased relative amounts of type V collagen have been reported in atherosclerosis (Ooshima, 1981). Type V collagen has the ability to bind to

insulin (Yaoi *et al.*, 1991) and to heparin/heparan sulfate (Richard *et al.*, 1989) with apparent higher affinity than collagen types I, II, III, IV, or VI, fibronectin, or laminin. Insulin bound to type V collagen has also been shown to retain mitogenic activity (Yaoi *et al.*, 1991) and heparin/heparan sulfate has been shown to modulate the biological activities of vascular endothelial cell growth factor (Lobb *et al.*, 1986) and basic fibroblast growth factor (Thornton *et al.*, 1983; Schreiber *et al.*, 1985). These findings suggest that type V collagen is important in the compartmentalization, storage, stabilization, and modulation of the activities of various growth factors. Since type V collagen also binds to thrombospondin (Mumby *et al.*, 1984), its interactions with thrombospondin and heparan sulfate may be important in the assembly of the ECM and in the regulation of its biological functions. Therefore, it is suggested that increased relative amounts of $\alpha 1(V)$ or type V collagen in the decidua provide a biochemical basis for functional regulation of the decidual cells.

The decidua is the specialized and highly differentiated endometrial stroma of pregnancy, and it is formed as a result of prolonged stimulation by oestrogen, progesterin, and growth factors, in particular, transforming growth factor- β (TGF- β) (Kauma *et al.*, 1990; Dungy *et al.*, 1991; Lea *et al.*, 1992; Vuckovic *et al.*, 1992) and platelet-derived growth factor (Surrey and Halme, 1991; Chegini *et al.*, 1992) generated by the trophoblasts of the implanting blastocyst and/or maternal platelets during invasion by the trophoblasts of the endometrium and disruption of its vessels. In particular, TGF- β has been reported to alter the synthesis and catabolism of matrix components and the expression of integrin (Roberts *et al.*, 1990, 1992; Border and Ruoslahti, 1992; Wahl, 1992). In conclusion, interstitial collagens, including type I, III, and VI collagens, were found to be localized diffusely in the stroma of the human endometrium during the menstrual cycle and in the decidua. Decidualization of the human endometrium may be characterized by increased relative amounts of type V and type I collagens and pericellular accumulation of basement membrane components, such as type IV collagen and laminin, around the decidual cells. The results of the present study provide insight into the hormonal regulation of the ECM, suggesting that alterations in the distribution and the composition of the ECM during the process of decidualization play an important role in implantation, invasion of trophoblastic cells and the maintenance of pregnancy.

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