Immunofluorescent localization of collagen types I, III, IV, V, fibronectin, laminin, entactin, and heparan sulphate proteoglycan in human immature placenta

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Abstract. The distribution of eight components of the extracellular matrix in immature human placenta was studied by an indirect immunofluorescence method with monospecific antibodies. In the stroma of the term chorionic villi, collagen types I, III, IV, V, and fibronectin formed a mesh of fibers and conglomerates. Heparan sulphate proteoglycan formed multiple conglomerates, whereas laminin comprised small, scanty, discrete granules. Collagen type IV, laminin, entactin, and heparan sulphate proteoglycan were confined to the basement membrane of the trophoblast. Sometimes, only collagen type IV was identified in fetal vascular basement membrane.
Key words. Immature placenta; extracellular matrix; immunofluorescence.

Placenta is widely used as a source of extracellular matrix (ECM), owing to its availability, accessibility and its wealth of ECM components [1, 2]. Despite its extensive use in biochemical studies, little is known of the distribution of specific ECM components in the placenta.

Initial immunomorphological studies have investigated the distribution of some ECM components in the placenta [3–5]. Amenta et al. have described the localization of collagen types I, III, IV, V, VI, fibronectin, and laminin in human term placenta [6]. Nevertheless, the location of the principal ECM components in the villous stroma and in the basement membrane (BM) of human placenta at an early stage of gestation still requires elucidation before the development of the human placenta can be understood.

This paper describes the distribution of eight components of ECM in human first-trimester chorionic villi, studied by indirect immunofluorescent technique using specific antibodies.

Materials and methods

8 human placentae (7–10 weeks of normal gestation) were obtained after therapeutic abortion. Polyclonal rabbit antibodies to collagen types I, III, IV, V, and fibronectin were obtained and characterized as previously described [7, 8]. Monoclonal rat antibodies to laminin, entactin and heparan sulphate proteoglycan were provided by Dr A. Ljubimoc (USSR Oncology Research Center) [9]. Control sections were treated with antibody to keratin (Daco, Danemark) or only with fluorescein isothiocyanate- (FITC)-conjugated antibodies. FITC-conjugated anti-rabbit and anti-rat antibodies were obtained from Cappel Laboratories (Westchester, PA). Tissue sample preparation and immunofluorescence staining were as described previously [10].

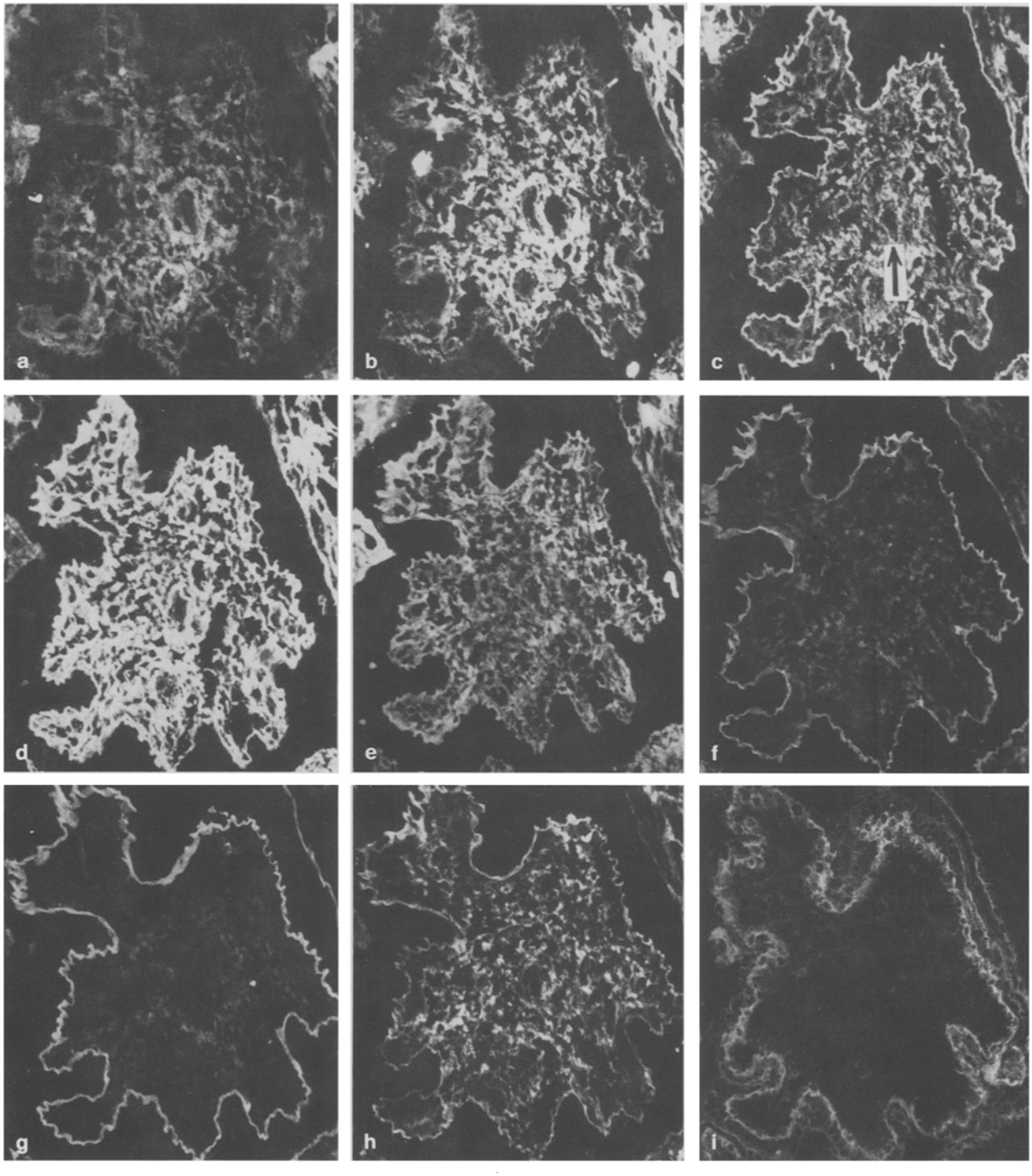
Results and discussion

In the villous stroma all collagen types and fibronectin were present in the form of a loose or fairly dense mesh of interwined fibers and conglomerates. More compact structures were observed around fetal capillaries (fig. a–e). Collagen type I occurred predominantly in the central regions of villous stroma (fig. a), whereas colllagen type III was detectable in the periphery also (fig. b). Collagen types I and III were sometimes uniformly distributed throughout the total area of the villi.

A significant amount of collagen type IV was always found in the villous stroma; this type was also observed within the trophoblastic BM and sometimes, indistinctly, in the fetal capillary BM, forming a thin interrupted line (fig. c, arrowhead).

Denser regions of collagen type V and fibronectin were observed throughout the villi, and also adjacent to trophoblastic BM (fig. d, e). Laminin was restricted to the trophoblastic BM, apart from small, sparse specks in the stroma (fig. f). Entactin showed immunoreactivity with the trophoblastic BM exclusively (fig. g). Heparan sulphate proteoglycan was detectable in the villous stroma as small to medium conglomerates. In this region it showed less immunofluorescence than in the trophoblastic BM, and it was not detectable in the capillary BM (fig. h). In the control section keratin was detected only in the basement region of the protoplasm trophoblastic cell (fig. i). On incubation with FITC-conjugated antibodies exclusively, no specific fluorescence was observed. None of the placental samples examined differed significantly with regard to the distribution of ECM components.

Because the immunofluorescence method gives only a relative idea of the quantitative ratio of the antigens identified we have described mainly the topography of ECM components. In our studies, all collagen types and fibronectin in the villous stroma showed a similar distribution and differed in the intensity of specific fluorescence. It is difficult to demonstrate at the light-microscopic level, the presence of collagen type V and fibronectin in the trophoblastic BM. A number of authors consider that fibronectin participates in the formation of the trophoblastic BM in the first trimester of gestation and subsequently disappears [5, 6].



Human placental villus at ten weeks of gestation. The indirect immunofluorescence method. Serial cryostat sections. Treatment with polyclonal antibodies to collagen types I, III, IV, V (*a, b, c, d*), fibronectin (*e*), monoclonal antibodies to laminin (*f*), entactin (*g*), heparan sulphate proteoglycan (*h*), and antiserum to keratin (*i*). x 96.
Collagens and fibronectin in the stroma are represented by a mesh of fibrils and conglomerates densely packed around the fetal capillary. Collagen Type IV, laminin, entactin, and heparan sulphate proteoglycan can be detected in the trophoblastic BM. Collagen type IV can also be detected in the fetal capillar BM (c, arrowhead). In the stroma these are occasional granules of laminin and moderate deposits and conglomerates of heparan sulphate proteoglycan. Keratin is detectable only in the protoplasm of trophoblastic cells (control section).

Our study has shown that trophoblastic BM contains collagen type IV, laminin, entactin and heparan sulphate proteoglycan components that predominate in the BM at any other location [11]. Immunohistochemically, however, it is known that the BM appears to be heterogeneous, and presumably so is the trophoblastic BM. Thus, merosin is a novel tissue-specific protein found in the BM of striated muscle, Schwann cells and trophoblast [12].

Fine, and sparse fragments of collagen type IV were revealed in the region of the fetal capillary BM, which accords well with the data obtained in the later gestation period, as compared with the trophoblastic BM[13]. Unlike other investigators[4,5], we detected typical BM components (collagen type IV, heparan sulphate proteoglycan and traces of laminin) in the villous stroma of the first-trimester gestation. Amenta et al. examined term placenta to assess the specific staining of collagen type IV and found traces of laminin in the villous stroma as rudimentary remnants of trophoblastic BM, that appeared as a result of continuous development of the organ[6]. However, the presence of components that were not specific to the basement membrane may account for a well-developed network of reticular ('argyrophilic') fibers in the villous stroma[14]. Besides interstitial types of collagen and fibronectin, reticular fibers of human lymph nodes have also been recorded as containing basic BM components[15]. Moreover, basement membrane macromolecules have been detected in human endometrial stroma during the menstrual cycle and early pregnancy[16]. This is consistent with the observation of Dallenbach-Hellweg who showed that reticular fibers are visible in the stroma from the middle of the proliferative phase and become more densely distributed towards ovulation[17]. It is interesting that collagen type IV, collagen of interstitial type and fibronectin participated in the formation of fibrosis villorum[10].

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Chymotrypsin-like and trypsin-like protease activities in the sea urchin (*Hemicentrotus pulcherrimus*) egg

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Abstract. Proteolytic activities in extracts of sea urchin eggs were examined using SDS (sodium dodecyl sulphate)-polyacrylamide gels. In the unfertilized eggs, proteases were detected as bands corresponding to the molecular weights of 40 kD and 26 kD on the gelatin gel, and 35 kD and 30 kD on the casein gel. Using various protease inhibitors, it was found that 40 kD, 30 kD, and 26 kD are chymotrypsin-like proteases and that 35 kD is a trypsin-like protease. The activity of the 40 kD chymotrypsin-like protease was found to be almost completely lost after insemination.
Key words. Sea urchin egg; protease; trypsin; chymotrypsin; fertilization.

It has been suggested that proteolytic enzymes play important roles in various aspects of animal development. Experiments with protease inhibitors indicated that some protease activities are involved in the process of meiotic maturation in starfish and mouse oocytes[1,2]. However, these proteases have not been extracted from fresh material. On the other hand, in sea urchin, the functions of egg proteases during fertilization, and their biochemical nature, have been well investigated. It was demonstrated that a trypsin-like protease in unfertilized sea urchin eggs contributed to the block of polyspermy caused by the elevation of the fertilization envelope and detachment of sperm from the egg surface[3-5]. The enzyme was purified and characterized[6,7]. Its activity was reported to be localized in cortical granules of unfertilized eggs, and released into the surrounding seawater by exocytosis soon