Participation of collagen types I, III, IV, V, and fibronectin in the formation of villi fibrosis in human term placenta

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With 3 Figures

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Summary

The indirect immunofluorescence method was used to study the human term placenta in pathological pregnancy for the distribution of collagen types I, III, IV, V, and fibronectin in fibrosis stromatis villi. All collagen types and fibronectin were shown to participate in fibrosis villorum formation. Fibronectin was also detected in the fibrinoid that surrounded villi at stroma. The presence of free cytotrophoblast cells in the fibrinoid was accompanied by a noticeable increase in fibronectin fluorescence. A significant amount of collagen types IV and V and a less amount of collagen types I and III were identified.

1. Introduction

In recent years, a series of publications have concerned the localization of extracellular matrix components in human placental villi both at early stages of development and by the moment of delivery (ISEMURA et al. 1985; KUROSAWA et al. 1985; YAMAGUCHI et al. 1986; AMENTA et al. 1986; YAMADA et al. 1987).

The only study available on pregnancy pathology reports the identification of collagen type IV and laminin in thickened basement membrane in pre-eclampsia (RISTELI et al. 1984). Since fibrosis villorum is a typical demonstration of the placenta’s “aging” and various pathological processes (FOX 1978), a question arises to what extent the process possesses the general features typical of sclerosis in other organs and tissues.

In the present study, the immunofluorescence method was used to study the distribution of collagen types I, III, IV, V, and fibronectin in fibrosis villorum and in the fibrinoid around the villi in pregnancy complicated by toxicosis, pre-eclampsia, hypertension, and anemia.

2. Materials and methods

11 term placentae in pregnancy complicated by toxicosis (4 cases), pre-eclampsia (1 case), hypertension (4 cases), and heavy anemia (3 cases) were examined. Tissue samples were cut from the central (4 samples) and paracentral (4 samples) placental regions within 3 h after delivery. 2 samples from each region were frozen in liquid nitrogen. Serial cryosections were cut at 5 μm and fixed for 5 min in cooled acetone. The resting samples were fixed in 10% neutral formaldehyde and embedded in paraffin. Cryostat and paraffin sections were stained with hematoxylin-eosine, picrofudisin, and Masson’s trichrome stain.

The immunofluorescence was performed routinely in the indirect variant (STERNBERGER 1979). Antibodies to collagen types I, III, IV, V, and fibronectin were obtained and characterized as described earlier (SHEKHONIN et al. 1987). Serial cryosections were incubated with the antibodies at a concentration of 0.05 to 0.1 mg/ml for 1 h at room temperature, washed 3 times (10 min) in PBS (pH = 7.2) and then treated with FITC-conjugated rabbit antibodies.
Fig. 1. 40 week pregnancy placenta. Anemia and placenta previa are observed. Indirect immunofluorescence method. Serial cryostat sections. Collagen types I, III, IV, V (a, b, c, d); fibronectin (e); hematoxylin-eosin (f). Intensive fluorescence of collagen of all types in the stroma of villi. Uneven fluorescence of fibrinoid among the villi. Cytotrophoblast cells are not found. × 96.
Fig. 2. 40 week pregnancy placenta. Pre-eclampsia. Indirect immunofluorescence method. Serial cryostat sections. Collagen types I, III, IV, V (a, b, c, d); fibronectin (e); hematoxylin-eosin (f). Intensive fluorescence of fibrillar structures of big and small conglomerates. ×400.
Fig. 3. 40 week pregnancy placenta. Hypertension. Indirect immunofluorescence method. Serial cryostat sections. Collagen types I, III, IV, V (a, b, c, d); fibronectin (e); picrofuchsin (f). Intensive fluorescence of collagen of all types and fibronectin in the stroma of villi. Besides fibronectin, a significant amount of collagen type IV and V and a much less amount of collagen types I and III were identified around the villi in the fibrinoid. Cytotrophoblast cells are observed. ×96.
Participation and fibronectin in human term placenta

3. Results

Fibrosis villorum was detected in all the pathological cases examined. The villi appeared in immunofluorescence as homogeneous patterns containing a certain number of cellular elements. Besides, they lacked epithelial covering layer and stained pink with picrofudisin and blue with Masson’s trichrome stain.

The immunofluorescence study revealed thickened deformed gross coarse fibrillar structure, and conglomerates within the villi, which exhibited bright homogeneous fluorescence. It is noteworthy that the pattern of the fluorescence shown by antibodies to collagens appeared to be very similar in character and intensity to that of fibronectin (Fig. 1, 3). At higher magnifications, however, the described picture was more clearly visible on serial cryosections of separate fibrosed villi (Fig. 2). Tinctorial properties and content of the fibrinoid were mainly defined by the presence of free cytotrophoblast cells. The absence of the latter resulted in the fibrinoid’s yellow staining with picrofudisin and a red one with Masson’s trichrome stain. If treated with antibodies to fibrinectin, the fibrinoid exposed uneven fluorescence (Fig. 1e), being practically dark if treated with antibodies to collagens (Fig. a, b, c, d).

By contrast, the free cytotrophoblastic cells were accompanied by the fibrinoid’s pink staining with picrofudisin and a blue one with Masson’s trichrome stain. Besides, the fibrinoid exposed weak fragmental fluorescence if treated with antibodies to collagen types I and III (Fig. 3a, b); it contained a significant amount of collagen types IV and V (Fig. 3c, d), and it showed the most intensive fluorescence if treated with antibodies to fibronectin (Fig. 3e).

4. Discussion

The immunofluorescence method gives a relative idea of the localized antigens’ content and ratio. Nevertheless, we have obtained the data showing that collagen types I, III, IV, V, and fibronectin are actively involved in the formation of fibrosis villorum. In this respect, the fibrosis villorum differs from the similar sclerotic process in both wound healing (JACKSON 1982) and atherosclerosis (SHEKHONIN et al. 1987) characterized by the accumulation of predominantly interstitial collagen types. At the same time, collagen type IV was identified in the glomerular focal sclerosis areas of the kidney (STRIKER et al. 1984), along the sinuses in hepatic cirrhosis (MARTINEZ-HERNANDEZ 1985), and in some tumour stroma (SMIRNOV et al. 1985; NANAEEV et al. 1985). The conclusion was drawn that under pathological conditions, collagen type IV (a basement membrane’s major component) may be accumulated out of the basement membrane, thus participating in sclerosis development. It is of interest, however, that collagen type IV was identified in the stroma villi of full-time normal pregnancy (AMENTA et al. 1986). Presumably, the peculiarities of fibrosis villorum is related to that of placenta development and function. As a result of thorough examination, collagen type IV and V and a small amount of collagen type I and III were identified in the fibrinoid only in the presence of cytotrophoblast cells. Therefore there are strong reasons to believe that cytotrophoblast cells are responsible for collagen synthesis and accumulation as it was already proposed for fibronectin (YAMADA et al. 1987). This assumption well accords with the fact that amniotic epithelial cells originating from cytotrophoblasts are capable of synthesizing collagen types III, IV, V, and laminin in vitro (ALITALO et al. 1980). It has been shown that a long-term cultivation of cytotrophoblasts leads to the appearance of collagen type IV (MORGAN et al. 1985). Thus, we may assume that it is synthesetical activity that partly contributes to the formation of fibrosis villorum.

Literature


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