NEGATIVE SURFACE CHARGE OF ABNORMAL HUMAN TROPHOBLAST

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In order to characterize the negative surface charge of the abnormal human trophoblast and to cast light on its biological role, the negative charges on the trophoblastic surface of spontaneous abortion, partial mole and complete mole were investigated by electron microscopy using cationized ferritin as an ultrastructural marker. The degree and distribution of negative charge were also studied in comparison with surface ultrastructure and surface glycoprotein identified by ruthenium red staining.

In a case of spontaneous abortion, the surface structure of the human trophoblast differed from site to site, correlating best with the degree of degeneration of the syncytiotrophoblast itself. For villi from a spontaneous abortion, which were otherwise normal in routine electron microscopy, the surface glycoprotein was observed to make prominent aggregates on the trophoblastic surface in concomitance with the reduced negative surface charge. In a severely degenerated system, the basic structure of the surface glycoprotein was lost in accordance with significant decrease of the negative surface charge. This finding seems to show the possible role of the negative surface charge of the trophoblast, that with the electrostatic repulsive forces, the membrane-bound glycoproteins can maintain their normal regular positions and, as a result, membrane specificity of the trophoblast can be expressed.

When both complete hydatidiform mole and partial mole were observed by electron microscopy using ruthenium red staining and cationized ferritin, the complete mole differed essentially from the partial mole in that the former resembled normal villi while the latter resembled spontaneous abortion.

The trophoblastic surface mucin has recently received special interest from researchers in the field of reproductive physiology (2, 4, 5, 6, 7, 9, 17, 18, 19). The mucin is considered to play an important role in blastocyst attachment to the endometrium (18), and to prevent rejection of the fetus by the mother via a masking of the fetal surface antigen. Currie and Bagshawe (8) presented the attractive hypothesis that the peritrophoblastic surface coat of sialomucin bears a high electronegative charge and escapes attacks from maternal lymphocyte by electrochemical repulsion. In these aspects, the basic structure and the character of the negative surface charge of the normal human trophoblast have been studied with the discussion on its biological role (13, 14).

In the present study, the negative charge on abnormal trophoblastic surface was

studied using cationized ferritin as an ultrastructural marker. The relationship between negative surface charge and surface glycoprotein was also examined by ruthenium red staining of the surface glycocalyx. The differences of surface ultrastructure, and the degree and distribution of negative charge of the trophoblast among spontaneous abortion, partial mole and complete mole were studied, with special references to their biological implications.

MATERIALS AND METHODS

Various abnormal human placental villi were obtained from 15 cases of spontaneous abortion, 2 cases of partial mole and 2 cases of complete hydatidiform mole. The tissue was dissected into small pieces and treated as soon as possible with the following procedures.

Routine Electron-microscopy

The tissues for transmission electron microscopic study were fixed with modified Karnovsky's fixative (2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3), post-fixed with 1% osmium tetroxide, dehydrated in graded ethanol and embedded in Epon-araldite. Ultrathin sections, cut on an LKB 8800 electron microtome, were stained with uranyl acetate and lead citrate. Observations were made with a H-700 electron microscope (Hitachi Co., Japan).

Ruthenium Red Staining

The tissues to be stained with ruthenium red were fixed for one hr in a mixture of the following solutions: 0.5 ml of 3.6% glutaraldehyde, 0.5 ml of 0.2 M cacodylate buffer (pH 7.3) and 0.5 ml of ruthenium red stock solution (1,500 ppm in water). After washing in two to three changes of 0.1 M cacodylate buffer the tissues were post-fixed for 3 hr in a mixture of 0.5 ml of 5% osmium tetroxide, 0.5 ml of 0.2 M cacodylate buffer (pH 7.3) and 0.5 ml of ruthenium red stock solution (15). They were rinsed briefly with the buffer, dehydrated with the standard ethanol series and embedded in Epon-araldite.

Labelling Procedure with Cationized Ferritin

The tissue was washed in pH 7.3 Veronal/HCl-buffered NaCl solution (buffered saline), and put into a solution consisting of 0.1 ml of cationized ferritin (Miles-Yeda Ltd., Israel) and 0.4 ml of Veronal/HCl-buffered saline for 30 min after fixation with modified Karnovsky's fixative for 5 min. The tissues were washed in two to three changes of buffered saline and then treated with the routine methods for electron microscopy.

RESULTS

Placental surface of spontaneous abortion

In a case of spontaneous abortion, the surface structure of the human trophoblast differed from site to site. The factor which correlated best with the structural changes of the trophoblastic surface was not the pre-operative urinary human gonadotropin (HCG) level but the degree of degeneration of the syncytiotrophoblast itself. Where the syncytiotrophoblast showed no degenerative change, the number, size and figure of the microvilli and micropinocytic pits were almost normal in com-

parison with the villi of corresponded gestational age. If the syncytiotrophoblast exhibited a degenerative appearance, such as a vacuolated rough endoplasmic reticulum with degranulation of the membrane-bounded polyribosomes, increased lipid droplets, a loosened matrix, a chromatin condensed nucleus with enlarged nuclear pores and so on, then the surface structure became destructive and degenerative. For example, in a mild case the microvilli became thinner, were partly broken, or decreased in number; in a severe case, the microvilli were no longer discernible and the plasma membrane was completely destroyed, and as a result, the intracellular organelle, the matrix or nucleus were exposed directly on the cell surface.

For a mild case of degeneration the ruthenium red staining method revealed the basic preservation of two different adjacent parts consisting of ruthenium red positive thicker and thinner layers. However, unlike the trophoblastic surface of the normal placenta, the thinner parts seemed to decline in some case and it was difficult to identify the ruthenium red positive layer in the so-called thinner layer between the thicker ones. Instead, the thicker parts became much more prominent and as a result the basic structure of the surface glycoprotein of the human trophoblast was clarified. It consisted of ruthenium red positive aggregates, from 15 to 25 $m\mu$ in height spaced 50 to 100 $m\mu$ away from neighboring aggregates (Figs. 1, 2). In a severely degenerated system, it was difficult to identify not only the basic structure of the surface glycoprotein but also the ruthenium red positive layer itself.

In a case of spontaneous abortion, the cationized ferritin particles on the trophoblastic surface generally decreased in number. Where the syncytiotrophoblast

FIG. 1. Trophoblastic surface glycoprotein of spontaneous abortion, demonstrated by ruthenium red staining. Orig. Mag. $\times 30,000$

FIG. 2. Basic structure of surface glycoprotein of human trophoblast.

exhibited a mildly degenerative appearance, the basic distribution pattern, that is, two different configurations as thicker and thinner layers were almost the same as that seen in the normal placenta of corresponding gestational age. In the severely degenerative syncytiotrophoblast, ferritin particles were significantly fewer in number and lost the basic pattern of two configurations on the cell surface (Fig. 3). In these cases the ferritin particles were not evenly distributed in a set manner on the cell surface.

FIG. 3. Negative surface charge of severely degenerative trophoblast in a case of spontaneous abortion.
Orig. Mag. $\times 40,000$

Surface structure and negative surface charge of partial mole

In general, the surface structure of a partial mole did not differ macroscopically between the normal and molar parts. The surface structures were nearly the same as those of a spontaneous abortion. The syncytiotrophoblast showed moderate to marked degenerative changes: i.e. an increased number of lipid droplets below the cell surface, a loosened matrix and vacuolated cisterna of rough endoplasmic reticulum with degranulation. In accordance with these changes, the trophoblastic surface was ordinarily destroyed with the loss of microvilli. The area stained by the ruthenium red decreased and, in some areas, the destruction of the surface structure was so severe that it was almost impossible to find the ruthenium red positive layer. The distribution pattern of the negative surface charge which was demonstrated by cationized ferritin was almost the same as that from spontaneous abortion. Generally, for the case of a partial mole there was a marked decrease in the number of cationized ferritin particles on the trophoblastic surface.

Surface structure and negative surface charge of complete mole

Both normal early placenta and hydatidiform mole showed similar ultrastructural features in the trophoblastic layer. Their outer surface consisted of syncytiotrophoblasts which were multinucleated giant cells with marginal condensed chromatin, having on their surface numerous slender but sometimes thickened, short, blunted and bulbously expanded microvilli with fibrillary core, multiple pinocytotic and coated vesicles below the cell surface, well developed canalicular systems such as rough and smooth endoplasmic reticulum and Golgi complex and small rounded

FIG. 4. Negative surface charge of the complete mole, almost identical with that of normal villi of corresponded gestational age. Orig. Mag. $\times 25,000$

mitochondria. For the complete hydatidiform mole, the microvilli were more prominent and irregular with more bulbously expanded tips than normal as was confirmed by both transmission and scanning electronmicroscopy. The distribution pattern of surface glycoprotein, stained with ruthenium red, was almost the same as that from a normal pregnancy of corresponding gestational age, and basically, consisted of the thicker and thinner ruthenium red positive layers.

The total number of ferritin particles per unit length on the basal line of the trophoblastic surface was slightly increased in the complete mole in comparison with the normal villi of corresponding gestational age. When the tissue was prefixed with modified Karnovsky's fixative and then labelled with cationized ferritin, one to four layers of cationized ferritin particles adhered primarily to the cell surface distributing in two different configurations: i.e. the thicker and thinner ones. The distribution pattern and localization site on the cell surface were almost the same as those of the normal villi (Fig. 4).

DISCUSSION

The surface glycoprotein of the normal human trophoblast, demonstrated with ruthenium red staining, has been shown to consist of two different layers, a thinner and thicker one, which corresponded to the distribution of negative surface charge on the cell surface (3, 14). The most striking finding, shown in the present study, is that the surface glycoprotein distributed on the trophoblastic plasma membrane forming regularly situated aggregates, 15 to 20 $m\mu$ in diameter separated by 50 to 100 $m\mu$ from neighbouring ones.

In a material obtained from a spontaneous abortion, the ruthenium red staining reveals that the surface glycoproteins often make prominent aggregates in concomitance with a decreased negative surface charges. Though, whether it is only a result of the abortion or whether it induces the abortion, is not possible to determine, the negative surface charge of the placental villi in a case of spontaneous abortion decrease in absolute amount and as a result the membrane-bound glycoproteins can no longer maintain their normal position nor express the membrane specificity of the trophoblast.

By analysing the surface morphology and negative surface charge, distinct differences between partial mole and complete mole could be found. Both the molar part and macroscopically normal part of the partial mole resemble the placental villi of spontaneous abortion in that the total surface charge and membrane-bound glycoprotein changed according to the degree of degeneration of the underlying syncytiotrophoblast. On the other hand, though the molar vesicle of the complete hydatidiform mole contained a slightly stronger negative charge than the normal placental villi of corresponding gestational age, the distribution pattern of surface glycoprotein and negative surface charge were basically the same. These findings on the differences between partial and complete moles are in accordance with the recent reports concerning the nature of both moles (1, 10, 11, 12, 16, 20, 21, 22).

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