Immunohistochemical Location of Major Histocompatibility Complex Class 1 Antigens in Human Placenta

Yu. V. Gladyshev, S. I. Kolesnikov, and S. V. Alisievich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 2, pp. 225-227, February, 2000
Original article submitted June 7, 1999

Distribution of major histocompatibility complex class I antigens in the postpartum human placenta was studied by immunohistochemical method. Positive staining was observed in endotheliocyte cytoplasm in vessels of chorionic villi. The surface of trophoblast, cytotrophoblast, and connective tissue cells did not stain. These data indicate a peculiar «masking» of antigens essential for normal course of gestation.

Key Words: *placenta; major histocompatibility complex antigens*

Major histocompatibility complex (MHC) antigens are products of genes from 3 locuses forming the main histocompatibility complex on the cell surface [7]. The role of MHC gene products are now well known. In humans, it was denoted HLA locus and subdivided in two large groups, ABC and DR. HLA antigens are expressed on nucleated cells and are closely connected to cell membrane [5].

HLA gene products participate in the recognition of their own and foreign antigens and are responsible for cell-to-cell interactions. They play the main role in allograft rejection and immune response [2]. HLA molecules are involved in antigen reception and processing by binding to T lymphocyte receptor; cytotoxic lymphocytes recognize antigens only in association with HLA molecules [3].

From immunological viewpoint, the fetus is an allotransplant, and therefore gestation can be regarded as an immunological paradox. Mechanisms of long-term protection of the fetus are well studied: the barrier role of trophoblast, impact of maternal blocking antibodies and suppressive humoral factors were demonstrated [6]. However, expression of HLA antigens in human placenta received little attention, though normal development of the fetus depeneds on the regulation of HLA expression on placental membranes.

We studied structural location of HLA antigens (ABC locus) in human placenta at the light optic and ultrastructural levels.

MATERIALS AND METHODS

Placental specimens containing all layers were collected immediately after spontaneous labor and fixed in 4% paraformaldehyde on 0.1 M phosphate buffer (pH 7.4) for 4 h. Material was dehydrated in alcohols and xylene and embedded in paraffin. Murine polyclonal antibodies to human HLA antigens (ABC locus) were used in immunohistochemical studies. Colloid gold particles were prepared as described previously [3]. Optimal amount of second antibodies (determined by Zsigmondy's test) was mixed with colloid gold, pH was adjusted to 8.0 with 0.2 M K_2CO_3, and the complex was centrifugated at 25,000*g* for 30 min. The precipitate was resuspended in 1 ml phosphate buffer containing polyethyleneglycol (mol. weight 20,000 D) in a concentration of 0.2 mg/ml.

After deparaffinization the sections were placed into phosphate buffer and incubated for 30 min in 1% bovine serum albumin and then incubated with antibodies to the HLA locus ABC diluted 1:100 (without washing) for 1 h at 37°C in a humidified thermostat.

Research Center of Medical Ecology, Research Center of Reconstructive and Repair Surgery, East Siberian Research Center, Siberian Division of Russian Academy of Medical Sciences, Irkutsk

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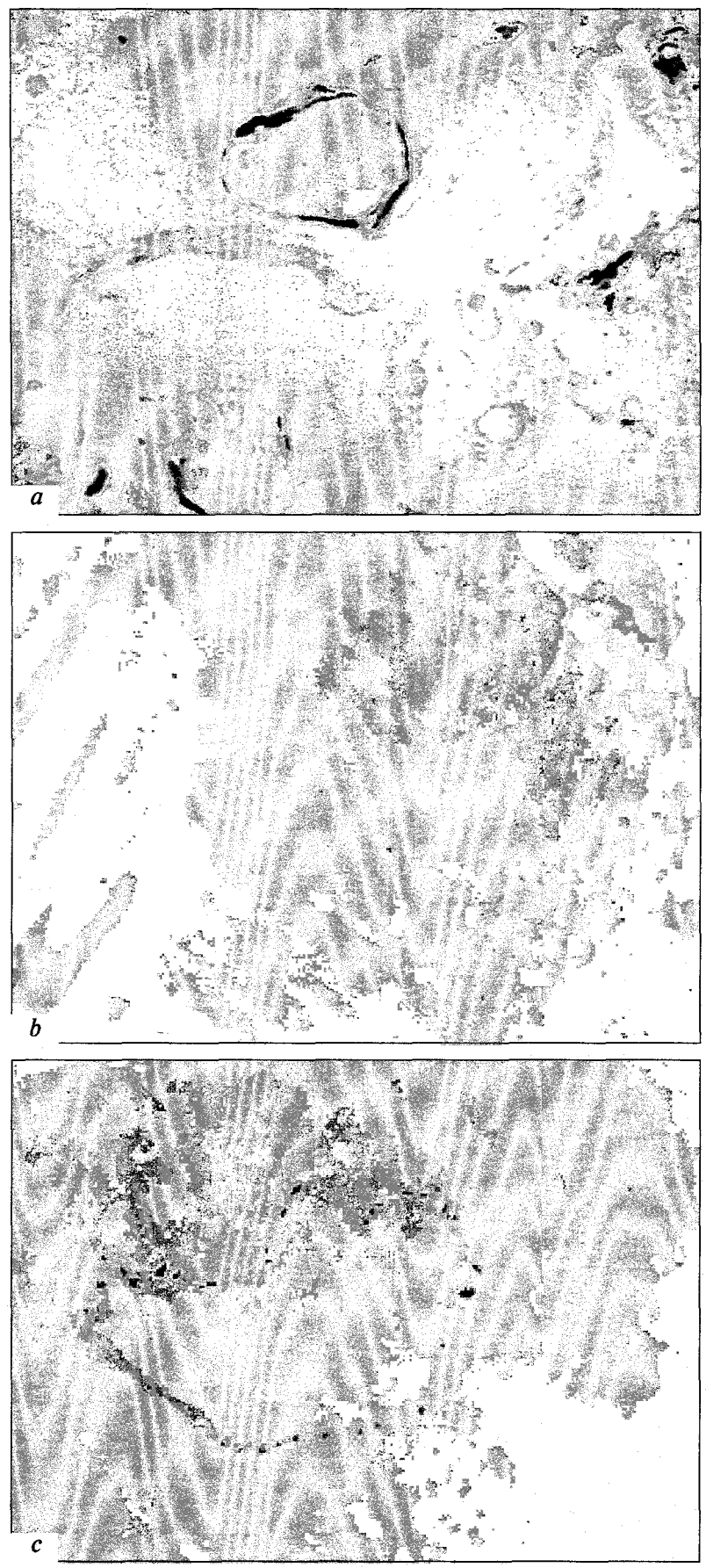

Fig. 1. Immunohistochemical location of HLA-ABC antigens in human placenta. *a*) staining of endotheliocyte cytoplasm of chorionic villi; *b*) colloid gold on the surface of endotheliocyte cytoplasm; *c*) colloid gold on the nuclear membrane of stromal cell. *a*) light microscopy, ×400; *b-c*) electronograms without additional staining, ×32,000.

After incubation, the samples were washed for 30 min in phosphate buffer to remove excess first antibodies, and second antibodies conjugated with colloid gold particles were layered and incubated for 2 h in a humidified chamber at 37°C for 2 h.

Some sections were washed with phosphate buffer, dehydrated, and without second staining embedded in balm. Other samples were dehydrated and embedded in epon-araldite routinely.

Sections not incubated with specific antibodies and sections treated with second antibodies alone served as the control. Ultrathin sections were made on a Reichert ultratome and examined under a Phillips electron microscope. For routine morphological studies, the sections were stained with hematoxylin and eosin.

RESULTS

Postpartum placenta stained with hematoxylin and eosin was characterized by typical structure. The villi were of different diameters with well-developed capillary and vascular network. Trophoblast is virtually homogenous; it is a symplast with scarce underlying cytotrophoblast cells. Intervillous space without signs of congestion contains different numbers of maternal erythrocytes.

Light microscopy of immunohistochemical preparations showed staining only of endotheliocyte cytoplasm of large, intermediate, and terminal villi (Fig. 1, *a*). Cytotrophoblast, syncytiotrophoblast, and connective tissue cells did not stain. At the ultrastructural level, colloid gold particles in the placenta were located on endotheliocyte surface (Fig. 1, *b*). In some cases stromal cell of villous chorion were stained (Fig. 1, *c*).

Structural location of HLA antigens, specifically of HLA class 2 antigens (DR), was studied in recent years. HLA-DR-positive cells were detected among placental macrophages and dendrite cells [8]. Location of HLA antigens on blastocytes, early human and animal embryos was investigated [2]. Immunological location of HLA-ABC antigens in human placenta has not been described, but summary pool of HLA antigens in human placenta homogenate has been studied by immunochemical methods. Some authors revealed no HLA molecules on the trophoblast [4], but this result can be caused by tissue preparation for immunochemical studies, because these antigens, being membrane proteins and glycoproteins, can be lost during isolation.

Our results indicate that the absence of HLA-ABC antigens on the surface of villous chorion trophoblast is the key factor of fetus protection in humans, explaining the absence of maternal reaction to MHC, because the surface facing maternal blood serves as a buffer because of its antigenic neutrality.

REFERENCES

1. G. Frens, *Nature,* **241,** 20-30 (1973).
2. J. R. Head, B. L. Drage, and F. A. Zuckerman, *Am. J. Reprod. Immunol.,* **15,** No. 2, 12-18 (1987).
3. D. H. Katz, *Cold Spring Harb. Symp. Quant. Biol.* (1977), pp. 611-624.
4. C. A. Labarrere, W. D. Falk, and O. H. Althabe, *Am. J. Reprod. Immunol.,* **21,** 16-28 (1989).
5. A. Mojsky, *Cas. Lek. Cesk.,* **128,** 152-153 (1989).
6. R. E. Rocklin, J. L. Kitzmiller, and M. D. Kaye, *Annu. Rev. Med.,* **30,** 375-404 (1979).
7. A. Sveagard, M. Hange, C. Jersild, *et al., An Introductory Survey. Monographs in Human Genetics,* Basel (1979), vol. 7, p. 230.
8. L. Sutton, D. Y. Masson, and W. G. Redman, *Immunology,* **49,** 103-112 (1983).