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Double Immunoelectron Microscopic Labelings of Human Chorionic Gonadotropin and Human Placental Lactogen in Human Chorionic Villi

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Abstract : Simultaneous immunoelectron microscopic localization of human chorionic gonadotropin (HCG) and human placental lactogen (HPL) was examined on the same ultrathin section of human chorionic villi by means of the double labeling technique. Using specific rabbit antisera against HCG and HPL followed by goat anti-rabbit IgG-coated colloidal gold of different sizes (15 nm and 5 nm), immunoreactions of HCG were concentrated on middle-sized secretory granules of 200–300 nm and large dense bodies of 500–1000 nm, while those of HPL were exclusively located on small secretory granules of 80–180 nm. The present experiment provides direct evidence for our previous data that HCG and HPL seem to be stored in different granular components in the syncytiotrophoblast.

Key words : human chorionic villi, syncytiotrophoblast, HCG, HPL, double immunoelectron microscopy.

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Introduction

Human chorionic villi synthesizes and secretes several important endocrine hormones into the maternal blood. Among them, human chorionic gonadotropin (HCG) and human placental lactogen (HPL, somatomammotropin) are two major hormones which are essential for the maintenance of pregnancy. Many immunocytochemical studies at electron microscopic level have been conducted to detect the immunoreactive sites of HCG (Dreskin *et al.*, 1970; Hamanaka *et al.*, 1971; Genbacev *et al.*, 1972; Hayashi, 1980; Morinaga *et al.*, 1984) and HPL (Hayashi, 1980). We also have reported immunoelectron microscopic localization of HCG (Fujimoto *et al.*, 1986) and HPL (Hamasaki *et al.*, 1987), separately. In our previous papers, HCG and HPL were shown to be located on different sized granular components in the syncytiotrophoblast.

The purpose of the present experiment was to obtain direct evidence of simultaneous localization of HCG and HPL in the same ultrathin section by means of the double immunoelectron microscopic labeling technique.

Materials and Methods

Tissue preparation

Four human placentas of 9 weeks of gestation obtained from both natural and therapeutic abortions were fixed in Zamboni solution (Zamboni & De Martino, 1967) for 18 hr at 4°C. Specimens were washed with 0.1 M phosphate buffer containing 10% sucrose (pH 7.4) for 2 hr at 4°C, dehydrated in graded concentrations of acetone, and embedded in epoxy resin.

Immunoelectron microscopy

The protein A-gold technique (Ueda & Fujimoto, 1984) was carried out for the separate labeling of HCG and HPL. For the double labelings, the method of Bendayan (1982) was used with some modifications. Ultrathin sections mounted on uncoated 150-mesh nickel grids were placed, the first face (face A) down, on drops of 0.1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 15 min. Face A was then incubated with the first antibody, rabbit anti-human chorionic gonadotropin (anti-HCG) or rabbit anti-human placental lactogen (anti-HPL) (DAKO Immunoglobulins, Denmark) for 2 hr. Both antisera were diluted at 1:1000 by 0.1% BSA-PBS. After rinsing by successively floating on several drops of PBS, face A was then transferred to goat-anti-rabbit IgG-coated colloidal gold of 15 nm (GAR-G15; mean size 14.9 nm, standard deviation 1.3 nm; Janssen Life Science Product, Belgium) which was diluted 1:100 by 0.1% BSA-PBS for 1 hr. The grids were completely washed in PBS and distilled water. After drying, the grids were turned over to the second face (face B) down, and placed on drops of 0.1% BSA-PBS for 15 min. Face B was incubated with the second antibody, HPL or HCG diluted at 1:1000 for 2 hr, rinsed on several drops of PBS, and conveyed to goat-anti-rabbit IgG-coated colloidal gold of 5 nm (GAR-G5; mean size 5.1 nm, standard deviation 1.2 nm; Janssen) which was diluted 1:50 for 1 hr. The grids were thoroughly washed in PBS, rinsed in distilled water, and dried. The sections were stained with 5% uranyl acetate for 5 min and examined with a JEM 100 CX electron microscope. For control purpose, normal rabbit serum or PBS was substituted for the specific antisera.

Results

As in our previous papers (Fujimoto *et al.*, 1986; Hamasaki *et al.*, 1987), it was seen that the syncytiotrophoblast of human chorionic villi at 9 weeks of gestation contains three kinds of membrane-bounded granular inclusions; small granules (SG) of 80–180 nm in diameter, middle-sized granules (MG) of 200–300 nm, and large bodies (LB) of 500–1000 nm (Fig. 1).

The immunoreactivity of HCG revealed by the protein A-gold technique was observed on the MG and LB but not on the SG (Fig. 2A). In contrast, the gold-labeled anti-HPL

was only located on the SG (Fig. 2B).

Figure 3 shows simultaneous localization of HCG and HPL. The first face of this section was incubated with anti-HCG serum followed by the GAR-G15, and then the second face was exposed to anti-HPL serum labeled by the GAR-G5. The large gold particles (15 nm) were concentrated on the MG and LB, while the small gold particles (5 nm) were mainly localized on the SG (Figs. 3A and 3B). Figure 4 illustrates the double immunostaining of anti-HPL with the GAR-G15 and anti-HCG with the GAR-G5. The HPL immunoreactivity was exclusively located on the SG with the large gold particles (Fig. 4A), whereas the HCG immunoreaction was localized on both the MG (Fig. 4A) and LB (Fig. 4B) with the small gold particles. There were no co-localizations of the large and small gold particles on the same granular component, and no differences in the present dou-

Fig. 1. A syncytiotrophoblast (ST) of human chorionic villi at 9 weeks of gestation fixed with Zamboni-osmium tetroxide, and stained with uranyl acetate and lead citrate. Three kinds of granular components, small granules (SG), middle-sized granules (MG), and large bodies (LB) are seen in the cell. G: Golgi apparatus, LD: Lipid droplets. Scale, 1 μ m.

ble immunostaining pattern between natural and therapeutic abortion specimens. In the control sections, no specific localization of gold particles of either size was observed.

Discussion

We have suggested in our previous studies that HCG and HPL were separately distributed in different-sized granular components in the syncytiotrophoblast (Fujimoto *et al.*, 1986; Hamasaki *et al.*, 1987). In spite of the difference in the biochemical nature of HCG and HPL (glycoprotein and simple protein, respectively), it is difficult to deny completely the possibility of co-existence of both hormones in the same granule. The present double immunoelectron microscopic labeling technique first demonstrated the simultaneous local-

Fig. 2. Immunoelectron microscopic localizations of HCG (A) and HPL (B) in a syncytiotrophoblast. The protein A-gold labeled HCG immunoreaction is observed on middle-sized granules (MG) and a large body (LB) (A). In contrast, the protein A-gold labeled HPL immunoreaction is exclusively seen on small granules (SG) (B). Scales, 0.5 μ m.

ization of HCG and HPL in the same ultrathin section. When either anti-HCG serum was applied to the first face or to the second one, the immunoreactivity of HCG occurred preferentially in the middle-sized granules and the large bodies. *Vice versa*, the immunoreaction of HPL was exclusively present in the small granules.

In the present experiment, we confirmed separate localizations of HCG and HPL using Bendayan's two-face method (Bendayan, 1982), since we did not observe co-localization of the large and small gold particles on the same granular component. However, the use of two specific antisera raised in the same species, i. e. rabbit, also involved some risks of the artificial co-distribution of the large and small gold particles in the same inclusions. It is preferable to use not only two separate antisera from different species but also two different-sized colloidal golds conjugated with different species' immunoglobulins.

In our previous paper, we mentioned that both small granules and middle-sized granules are derived from the Golgi apparatus and released by exocytosis into maternal blood (Fujimoto *et al.*, 1986; Hamasaki *et al.*, 1987). The HCG-reactive large body is considered to be a reservoir site of this hormone (Hamasaki *et al.*, 1987). As we could not obtain a detailed fine structure concerning the Golgi apparatus in the present observation, it will be necessary to examine osmificated specimens to determine how these two kinds of granules

Fig. 3. Double immunoelectron microscopic labelings of HCG and HPL in a syncytiotrophoblast. The HCG immunoreactivity revealed by 15 nm gold particles is localized on middle granules (MG) and a large body (LB), whereas the HPL immunoreactivity displayed by 5 nm gold particles is present on small granules (SG). Scales, 0.1 μ m.

Fig. 4. Double immunoelectron microscopic labelings of HPL and HCG in a syncytiotrophoblast. Immunoreactions of HPL with 15 nm gold particles are observed on small granules (SG), while those of HCG with 5 nm gold particles occur on middle-sized granules (MG) and a large body (LB). Scales, 0.1 μ m.

are segregated from the Golgi apparatus. Moreover, the changes of serum and urine concentrations of HCG and HPL are quite different during the course of gestation; those of HCG represent high levels in the first trimester (Ashitaka *et al.*, 1974) and those of HPL reach a peak at the full term (Biswas *et al.*, 1972). More detailed double immunoelectron microscopic investigations are necessary to clarify the synthetic pathway of these hormonal contents as well as the time course changes of granular inclusions during gestation, and such studies are now in progress in our laboratory.

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二重免疫電顕標識法による HCG と HPL のヒト胎盤絨毛における局在

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要 旨: 二重免疫電顕標識法を用いてヒト胎盤絨毛における胎盤性腺刺激ホルモン (HCG) と胎盤ラクトジェン (HPL) の局在を, 同一超薄切片上で検索した。超薄切片の片面をウサギ抗 HCG 血清 (または抗 HPL 血清) と 15 nm 径金粒子標識ヤギ抗ウサギ免疫グロブリンで, 他面をウサギ抗 HPL 血清 (または抗 HCG 血清) と 5 nm 径金粒子標識ヤギ抗ウサギ免疫グロブリンでそれぞれ反応させ, 径の異なる金粒子の局在性を電顕にて観察した結果, HCG の抗原性は合胞体細胞内の 200-300 nm 径中型分泌顆粒と 500-1000 nm 径電子密小体に, HPL の抗原性は 80-180 nm 径小型分泌顆粒に限局することが明らかとなった。今回の二重標識実験によって, 合胞体細胞が HCG と HPL を別個の分泌顆粒として産生・放出するという従来の考察が証明された。

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