Immunoelectron Microscopy of Human Chorionic Gonadotropin (hCG) and Human Placental Lactogen (hPL) in the Syncytiotrophoblast of Human Placenta, Clarifying the Nature of the So-Called Large Dense Bodies

Kunshige HAMASAKI, Yoshiaki DoI*, Mitsuru YOKOYAMA**, Masamichi KASHIMURA and Sunao FUJITO

Department of Obstetrics and Gynecology, *Department of Anatomy, and **Laboratory of Electron Microscope, University of Occupational and Environmental Health, School of Medicine, 1-I, Iseigaoka, Yahatanishi-ku, Kitakyushu 807, Japan

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Abstract. The synthetic and secretory processes of human chorionic gonadotropin (hCG) and human placental lactogen (hPL) were investigated by immunoelectron microscopy which included the use of the double immunolabeling technique. The syncytiotrophoblast contains three kinds of granules: Small-sized granules (SG), middle-sized granules (MG), and large dense bodies (LB). Immunoreactions of hCG are localized in the MG and LB, while those of hPL are in the SG and LB. The double immunolabeling method proved without a doubt that hCG and hPL do not co-exist in the same SG or MG, but they are co-localized in the LB. This means that hCG and hPL are separately sorted into different secretory granules in the Golgi apparatus, but the LB are a kind of cytolysosome which are involved in the uptake and storage of both hPL and hCG.

Key words: Immunoelectron microscopy - hPL - hCG - Syncytiotrophoblast - Human placenta

INTRODUCTION

Recent immunoelectron microscopy has revealed that the syncytiotrophoblast of human chorionic villi is a site of synthesis and storage of human placental gonadotropin (hCG) and human placental lactogen (hPL), which are essential for the maintenance of pregnancy1-5,6. Moreover, the SG and MG were apparently segregated in the Golgi apparatus as secretory granules which contained hPL and hCG, respectively1,5. However, the nature and origin of the LB is still uncertain, and the possibility of the co-existence of hPL and hCG in the SG or MG has not been ruled out.

The present experiment which included the use of the double immunolabeling technique was performed to investigate whether hCG and hPL are co-localized in both kinds of secretory granules, and to investigate what role the LB plays in the syncytiotrophoblast.

MATERIALS AND METHODS

Human placentas from natural abortions at 7, 9 and 12 weeks of gestation were fixed in a Zamboni solution6 for 18 hr at 4°C. Half of the specimens were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hr at room temperature. After fixation, specimens were dehydrated in graded concentrations of acetone and embedded in epoxy resin. Ultrathin sections were made on a Sorvall MT-1 microtome with a diamond knife and collected on uncoated nickel grids. For conventional electron microscopy, the grids were stained with 5% uranyl acetate for 5 min and examined at a magnification of 1,000–15,000 times with a JEM 100 CX electron microscope at an accelerating voltage of 80 kV. For the light microscopic immunohistochemistry of the hCG and hPL, the unlabeled antibody peroxidase-antiperoxidase (PAP) technique was performed in paraffin-embedded sections. For immunoelectron microscopy, ultrathin sections of gold interference
colors were mounted on uncoated nickel grids, and the etching treatment with saturated sodium metaperiodate was carried out on the osmium-postfixed specimens only, to restore the antigenicity of the immunoreactions. The grids were placed on a drop of 0.1% bovine serum albumin (BSA) to minimize non-specific adsorption, then immunoreacted with rabbit anti-human hCG or hPL serum (DAKO Immunoglobulins, Denmark) with a dilution of 1:100 of 0.1% BSA-phosphate buffered saline (PBS). After rinsing with PBS, the grids were floated for 1 hr on a drop of colloidal gold particles (15 nm in diameter, mean diameter 14.9 nm, standard deviation 1.3 nm) conjugated with goat-anti rabbit IgG (GAR-G15, Janssen) diluted 1:100 in 0.1% BSA-PBS for 1 hr. The grids were stained with 5% uranyl acetate for 5 min and examined with a JEM 100 CX electron microscope. For control purposes, normal rabbit serum or PBS was substituted for the specific antisera.

For double immunolabeling, the method of Ben-Dayan was utilized with slight modifications. Ultrathin sections mounted on nickel grids were placed, the first face (Face A) down, on drops of 0.1% BSA-PBS for 15 min. Face A was then incubated with anti-hCG for 2 hr. Both antisera were diluted at 1:1,000 with 0.1% BSA-PBS. After rinsing with PBS, face A was transferred to the GAR-G15 diluted 1:100 with 0.1% BSA-PBS for 1 hr. The grids were stained with 5% uranyl acetate for 5 min and examined with a JEM 100 CX electron microscope. For control purposes, normal rabbit serum or PBS was substituted for the specific antisera.

DISCUSSION
We have previously suggested that hPL and hCG are separately sorted into SG (80–180 nm in diameter) and MG (200–300 nm in diameter) in the Golgi apparatus of the syncytiotrophoblast and that both kinds of granules are released from the apical cell surface into the chorionic space by exocytosis. The present experiment which includes the use of the double immunolabeling technique reconfirms our previous data and shows the impossibility of the coexistence of hCG and hPL in either SG or MG.

On the other hand, the LB (500–1,000 nm in diameter) shows immunolocalization of both hCG and hPL. The nature of the LB is still uncertain. Judging from their size and ultrastructure, they are not considered to be secretory granules. HAMASAKI et al. have determined the presence of iron in the LB by X-ray microanalysis.

The present double immunolabeling method re-