Immunodetection of Living Trophoblast

David Peleg MD[1], Aviva Peleg MSc[2] and Eliezer Shalev MD[3]

[1]Department of Obstetrics and Gynecology and [2]Research Laboratories, Poriya Government Hospital, Tiberias, and [3]Department of Obstetrics and Gynecology, HaEmek Hospital, Afula, Israel

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Abstract

Background: Human chorionic gonadotropin, the pregnancy hormone, is synthesized by trophoblast cells which make up the placenta.

Objective: To determine whether antibody to hCG can be used to specifically detect living trophoblast *in vitro* by binding to the external membrane.

Methods: Trophoblast was isolated from fresh placentas of women undergoing termination of pregnancy in the first trimester and incubated with monoclonal antibody to hCG. Anti-mouse immunoglobulin G with a fluorescent marker was then added.

Results: Syncytiotrophoblast stained positive on the external surface of the cell, while controls of leukocytes, endometrial cells and hepatocytes were negative.

Conclusion: The hCG monoclonal antibody may be used to specifically detect hCG on the surface of living trophoblast *in vitro*.

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Serum human chorionic gonadotropin testing, ultrasound examination, and curettage constitute the arsenal in the diagnosis of failed pregnancy. hCG testing is performed to determine the presence of pregnancy, and ultrasound and curettage to determine its location. Whereas the presence of trophoblast at curettage is diagnostic of an intrauterine pregnancy, its absence increases the possibility of an ectopic pregnancy. Curettings are often viewed under the microscope, but the definitive diagnosis is dependent on pathologic examination.

The syncytiotrophoblast of the placenta synthesizes hCG, whose function is to sustain the corpus luteum of pregnancy and progesterone secretion. Although much is known about the synthesis and kinetics of hCG, the cellular secretion of this molecule is not well understood. Immunostaining of fixed tissue has identified hCG in cytoplasmic granules and throughout the microvilli of syncytiotrophoblast [1]. Antibody to hCG has been used with some success to demonstrate the presence of residual choriocarcinoma [2,3]

The physician is often unable to determine the presence of chorionic villi at the time of curettage. This may lead to a delay in diagnosis of missed abortion or ectopic pregnancy. As the first step to overcome this problem, we undertook this study to determine if antibody to hCG will specifically bind to the external surface of living trophoblast *in vitro*.

Materials and Methods

Six fresh placentas from 6–11 week pregnancy terminations were immediately placed in cold saline (4°C). The tissue was washed in cold Ham's F10 (Life Technologies, Gaithersburg, MD, USA) and then mashed on a glass plate. The mixture was allowed to settle and the liquid fraction centrifuged at 1,500 rpm for 5 minutes and washed twice. The pellet was suspended in 150 µl of Ham's F10 at 4°C. The cells were incubated for 30 min with 1:10 monoclonal anti-hCG (Biodesign International, Kennebunkport, ME, USA) and then washed three times. Anti-mouse IgG (1:15) conjugated with FITC (The Binding Site Ltd, Birmingham, UK) was then added for an additional 30 min followed by three washings. The cells were examined by wet mount using an ultraviolet microscope. Comparative slides were stained with Giemsa for cell identification and quality. Trophoblast without addition of anti-hCG served as the control. Other cells tested included endometrium, leukocytes, and hepatocytes similarly treated. As a positive control, fixated sections of placenta were incubated with both antibodies as above.

Results

The larger syncytiotrophoblasts were positive for fluorescent immunostaining [Figure 1b]. The identity of these cells was confirmed by light microscopy [Figure 1a]. The smaller cytotrophoblasts were much more numerous than the larger syncytiotrophoblast. Cytotrophoblasts were negative or below detection, although an occasional cell stained positive. Similar control experiments with endometrial cells, leukocytes and hepatocytes were all negative. Trophoblastic staining was negative if anti-hCG was not added before the fluorescent marker. Paraffin-fixated tissue was positive with a very strong intracellular signal in the syncytiotrophoblast.

hCG = human chorionic gonadotropin

IgG = immunoglobulin G

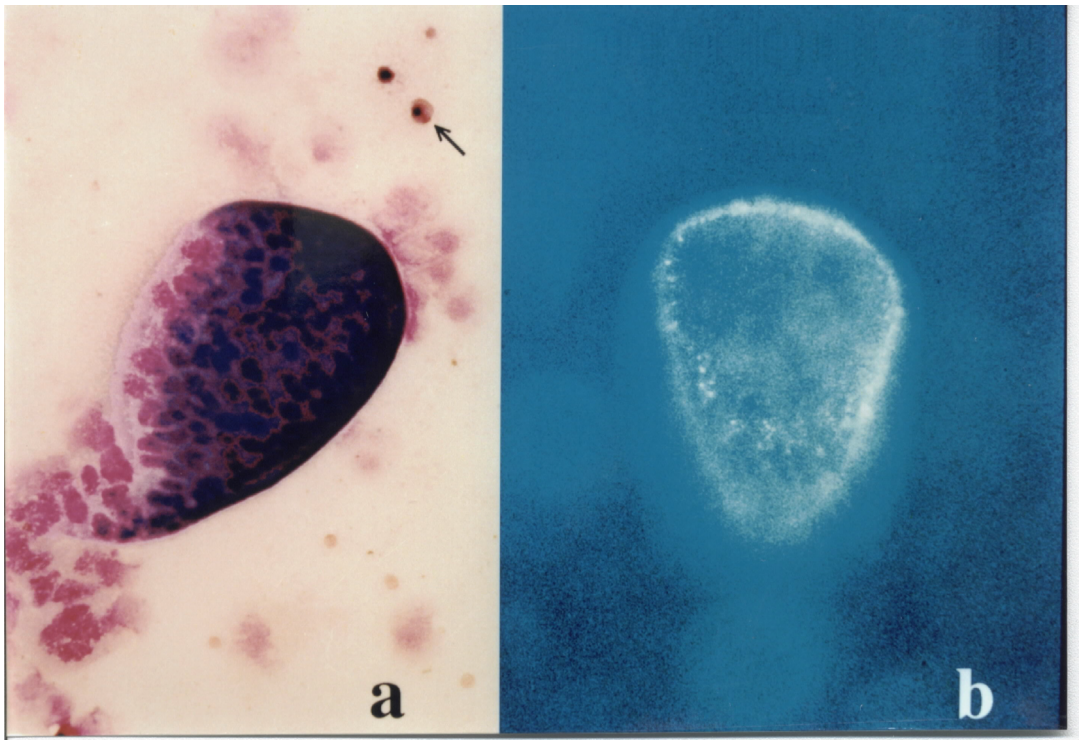

Figure 1. Anti-hCG binding to the surface of trophoblast. [**a**] Giemsa stain showing syncytiotrophoblast. Arrow points to fetal normoblast (12 μm diameter). [**b**] Positive immunofluorescent staining of anti-hCG on the external surface of a similar cell. Magnification x 400.

Discussion

Binding of anti-hCG to non-fixed trophoblast suggests the presence of hCG on the external surface of the cell membrane. This may be explained by two mechanisms. hCG has been shown to bind to receptors on human placenta [4], which has been proposed as the trigger for differentiation of syncytiotrophoblast from cytotrophoblast [5]. Alternatively, the monoclonal antibody may be capturing hCG or β-hCG as they are secreted. Non-specific permeabilization can be rejected as an explanation since there was no immunostaining if anti-hCG was omitted.

Although much is known about the genes coding for the two subunits of hCG (α and β), the regulation of their expression is only beginning to be understood. The α-subunit, common to the other glycoprotein hormones (luteinizing hormone, follicular-stimulating hormone, thyroid-stimulating hormone), is encoded by a single gene on chromosome 18. The β-subunit, specific for hCG, has a family of seven genes of which only three are expressible [6]. It has been suggested that placental gonadotropin-releasing hormone activates both the α and β genes possibly through a cyclic adenosine 3',5'-monophosphate second messenger [7,8]. After transcription and translation, the dimer is formed and non-covalently joined. Little is known about hCG secretion except that it may be packaged in cytoplasmic granules [1].

Whichever the mechanism, the ability to detect hCG on the surface of living hCG-secreting trophoblast can be advantageous. Several trials have used radiolabeled or conjugated antibody *in vivo* to detect and attack residual choriocarcinoma with va-riable results [2,3]. Specific *in vitro* binding to living trophoblast could be used for rapid identification of tissue at the time of curettage or removal of ectopic pregnancy. In these instances it is not uncommon to obtain tissue that does not display the typical villous pattern in suspension or under the microscope. In fact, we are attempting to develop an *in vitro* kit using a modification of the abovementioned technique to aid the physician in rapid identification of trophoblast.

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Correspondence: Dr. D. Peleg, Dept. of Obstetrics and Gynecology, Poriya Government Hospital, Tiberias 15208, Israel. Phone: (972-6) 673-8306, Fax: (972-6) 673-8487, email: peleg123@hotmail.com