

# Localization of the $\beta$ Chain of Human Chorionic Gonadotropin on Human Tumor Cells and Placental Cells<sup>1</sup>

Michael A. Naughton,<sup>2</sup> Deborah A. Merrill, Linda M. McManus, Louis M. Fink, Edward Berman, Miriam Jill White, and Antonio Martinez-Hernandez

Division of Perinatal Medicine, Departments of Pediatrics, Obstetrics-Gynecology [M. A. N., D. A. M., E. B., M. J. W.], and Pathology [L. M. M., L. M. F., A. M.], University of Colorado Medical Center, Denver, Colorado 80220

## SUMMARY

With the use of peroxidase-labeled antibody to the  $\beta$  chain of human chorionic gonadotropin, sections of ten human malignant tumors were found to react with this antibody. It is postulated that both selective host immunosuppression by tumors and selective maternal immunosuppression by fetal tissues may be mediated by human chorionic gonadotropin.

## INTRODUCTION

Previous work from this and other laboratories (2, 9, 17, 19, 30) has shown that HCG<sup>3</sup> inhibits the lymphocyte T-cell response in both MLC and phytohemagglutinin stimulation assays. These studies suggest that tolerance to the human fetus by the maternal immunological system is due, at least in part, to this inhibitory effect of placental HCG against immunologically competent maternal thymus-derived lymphocytes. Previous reports (5, 29) implicating cell surface mucoprotein complexes as immunoprotective shields in both normal and malignant trophoblast would also agree with this hypothesis. Increased urinary and serum levels of HCG have been reported in human patients with a variety of malignant tumors (4, 6, 12, 13). The purpose of this study was to ascertain the presence and localization of an HCG-like material in malignant cells of human tumors. Because the  $\alpha$  chain of LH and HCG are virtually identical, an antiserum to the  $\beta$  chain of HCG, which shares less

homology with LH, was chosen for localization of HCG in this study.

## MATERIALS AND METHODS

The  $\beta$  chain of HCG was localized on frozen sections or on tissue-cultured cells grown on glass slides by the indirect peroxidase-labeled antibody technique (25). Purified  $\beta$  chain of HCG (supplied by Dr. Vernon Stevens, Ohio State University School of Medicine, Columbus, Ohio) was injected into the popliteal lymph nodes of rabbits; the presence of antibodies was determined by immunodiffusion. The rabbit antiserum was passed through an acrylamide affinity column (20) containing HCG (Organon, Inc., West Orange, N. J.) to obtain specific anti- $\beta$  chain antibody. As has been previously shown (20), antibodies obtained by this procedure are electrophoretically pure and consistently show very low background. Sheep anti-rabbit antiserum was purified by an acrylamide affinity column containing rabbit  $\gamma$ -globulin and the specific antibodies were then conjugated (24) to horseradish peroxidase (type VI; Sigma Chemical Co., St. Louis, Mo.). Normal rabbit serum was used as a negative control. All antibodies and normal rabbit sera were absorbed with human spleen powder to reduce nonspecific absorption to the test tissues.

Placentae (8 weeks to term) and BeWo cells in culture [established from trophoblastic tumor cells of a postgestational human choriocarcinoma that synthesizes HCG (28), supplied by Dr. Roland Pattillo, Marquette School of Medicine, Milwaukee, Wis.] were used as positive controls.

Human malignant tumors were collected in the operating room or at necropsy, embedded in Ames O.C.T. (Ames Co., Miles Laboratories, Elkhart, Ind.), and quickly frozen using Dry Ice and alcohol.

## RESULTS

Normal rabbit serum controls of all tissues and BeWo cells were consistently negative. In placental tissue controls,

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<sup>2</sup> To whom requests for reprint should be addressed, at 4200 E. Ninth Ave., Denver, Colo. 80220.

<sup>3</sup> The abbreviations used are: HCG, human chorionic gonadotropin; MLC, mixed lymphocyte culture;  $\alpha$  and  $\beta$ , the 2 polypeptide chains of HCG and LH; LH, luteinizing hormone; BeWo, tissue-cultured cell line of human choriocarcinoma.

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the syncytiotrophoblast reacted positively with anti- $\beta$  chain antibody; a discrete, dark brown reaction product was present at the outer surface of the chorionic villi (Fig. 2a). The BeWo cells contained discrete positive cytoplasmic granules (Fig. 2b). Material reacting with anti- $\beta$  chain antibody was present in the malignant cells of 10 human tumors: 2 breast infiltrating duct carcinomas; 1 prostatic, 1 colonic and 1 pancreatic adenocarcinoma; 1 seminoma; 1 liposarcoma; 1 bronchogenic squamous cell carcinoma; 1 renal cell carcinoma; and 1 transitional cell carcinoma of the kidney. The reaction product in the malignant cells was localized in the cytoplasm with a more intense band of staining at or near the surface. Both stroma and normal tissue present in the sections were invariably negative (Fig. 1).

No tumor tissue was unequivocally negative; however, the results obtained with 1 Hodgkin's sarcoma, 1 malignant melanoma, 1 malignant hepatoma, 1 squamous cell carcinoma of the uterine cervix, and 1 endometrial stromal sarcoma were equivocal. This was due to 1 of several factors: the presence of other brown pigments (melanin and bilirubin), extensive necrosis resulting in nonspecific adsorption, or intrinsic peroxidase activity in the test tissues.

## DISCUSSION

The reason for maternal tolerance to placental tissues has not been adequately explained and is probably a multifaceted phenomenon. Since HCG is an inhibitor of the T-cell response (2, 9, 17, 19, 30), it is strongly suggestive that this substance could be partially responsible for nonrejection. However, the concentration of HCG required to produce complete inhibition of the T-cell responses to phytohemagglutinin and MLC is of the order of 10,000 IU/ml (2, 30), a concentration much higher than that found in sera of normal pregnant women. Evidence for the presence of HCG on the placental cell surface comes from work by others (10, 18, 22, 26) who used various methods of HCG detection and is confirmed by us (Fig. 2a) with anti- $\beta$  chain antibody. These studies demonstrate HCG as a continuous layer on the surface of the syncytiotrophoblastic cells. In addition, this study shows a similar layer of HCG-like material on the surface of 10 tumor cell types. This surface distribution of HCG is in contrast to that found with other hormones. For example, in rabbit and human pituitary tissue, LH is detected in discrete secretory granules, but no surface layer has been demonstrated for this or any other hormone (21, 23). If the function of HCG were solely hormonal, this unique surface distribution would be unexpected; however, the presence of HCG on the cell surface would provide the high concentration of HCG needed to inhibit the T-cell response.

The mechanism for retaining HCG on the surface of syncytiotrophoblast cells and of tumor cells is unknown, but it may reside in the final 30-amino acid sequence of the COOH-terminal end of the  $\beta$  chain (7). The structure of this "tail" region of the  $\beta$  chain of HCG, which is absent from the  $\beta$  chain of LH, is reminiscent of collagen and includes

several prolines and 5 serine-linked carbohydrate chains, structures not commonly associated with globular proteins. These characteristics may account for the ability of HCG to "stick" to the surface of the trophoblast and to tumor cells.

The presence of HCG in the serum and urine of patients with tumors arising from all embryonal layers has been reported (6, 12) and is currently under evaluation as a diagnostic test for neoplasia (31). The present work localizing the  $\beta$  chain of HCG in tumor cells indicates that this material is a product of the tumor cells rather than a host response. It is remarkable that a molecule that is present only in embryonal tissue should be produced by such a heterogeneous group of tumors. We would like to postulate that a parallelism exists between syncytiotrophoblast and tumors. Placental tissues are able to induce specific immune tolerance, and there is evidence that tumor tissues, too, evoke specific immune tolerance (16). Both placental and neoplastic tissues produce a T-cell-inhibiting substance resembling HCG, which may react with the T-cell to prevent maturation of the immune response. This mechanism could account for both the specificity of the immunosuppression in the mother during pregnancy and the recovery of her immunocompetence during the postnatal period. Such specific immunosuppression has been reported previously (8, 27). Those experiments showed that mothers who failed to respond with blastogenic transformation in MLC to the lymphocytes of their newborn sons had intermediate responses to the lymphocytes of their spouses and responded normally to the lymphocytes of another male. A similar mechanism might be operative when HCG is present on tumor cell surfaces specifically suppressing those T-cells capable of reacting with tumor antigens. The recent report of Fauve *et al.* (11) suggests that an immunosuppressive molecule produced by trophoblast and malignant cells is present in the mouse.

During oncogenesis, fetal genes are often expressed; examples are carcinoembryonic antigen (14), pancreatic embryonic antigen (3), and  $\alpha$ -fetoprotein (1). Such expression of fetal genes may also result in the production of HCG, one of the earliest embryonic genes to be expressed in ontogeny (15). The unique surface distribution of this molecule on the tumor cell could provide an effective means of masking the malignant cell from its host's immune surveillance system.

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Fig. 1. Six- $\mu\text{m}$  serial frozen sections of breast-infiltrating duct carcinoma. Group of malignant cells surrounded by dense stromal reaction. *a*, H & E,  $\times 72$ . *b*, anti- $\beta$  chain HCG rabbit antibody followed by peroxidase-labeled sheep anti-rabbit antibody. The tumor cells give a positive reaction particularly intense at the cell periphery. Stromal tissue does not stain.  $\times 72$ .

Fig. 2. Positive controls. Anti- $\beta$  chain HCG antibody followed by peroxidase-labeled sheep anti-rabbit antibody. *a*, 6- $\mu\text{m}$  frozen sections of 8-week placenta. Syncytiotrophoblast of chorionic villus contains reaction product. Nuclei appear negatively stained. A dense layer of reaction product covers the surface of these cells.  $\times 180$ . *b*, BeWo cell (48 h subcultured). The dense deposits of reaction product are present in discrete cytoplasmic granules. Adjacent small cells are negative.  $\times 180$ .

