Human Chorionic Gonadotropin in Human Neoplastic Cells

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Summary

Peroxidase-labeled antibody against the $\beta$ chain of human chorionic gonadotropin was used to demonstrate that 25 human malignant tumors contained this antigen. The antigen was localized both in the cytoplasm and on the surface of the malignant cells. Human chorionic gonadotropin may be responsible for both selective maternal immunosuppression by fetal tissue and host immunosuppression by tumors.

Introduction

It was previously reported by Adcock et al. (2) and Teasdale et al. (36) that HCG inhibits the thymic-derived lymphocyte response in both mixed lymphocyte culture and phytohemagglutinin stimulation assays. Similar results have been reported by Contractor and Davies (11), Kaye and Jones (23), and Han (18). Those experiments were done using commercial preparations of HCG with activities of about 3,000 IU/mg. Gunert et al. (17) and Caldwell et al. (8) reported that purified HCG has no activity on lymphocytes; however, Belling and Weksler (5) reported suppression of the mixed lymphocyte reaction by highly purified HCG (13,700 IU/mg) obtained by a different purification procedure. Because of these in vitro reactions, it seemed possible that some form of placental HCG may be involved in the tolerance of the human fetus by the maternal immunological system. Further attention was focused on this possibility because detectable urinary and serum levels of HCG have been reported in patients with a variety of malignant tumors (4, 7, 14, 15) and because neither fetuses nor tumors are rejected as foreign by their hosts. We have previously presented preliminary evidence (30) that material antigenically identical to the $\beta$ chain of HCG was present in 10 malignant tumors. This report indicates that, of the 28 human malignant tumors evaluated, 25 were positive with the enzyme-labeled antibody technique, including malignant cells of ectodermal, mesodermal, and endodermal origins.

Materials and Methods

The indirect peroxidase-labeled antibody technique (29) was used to localize the $\beta$ chain of HCG on 6-µm frozen sections or on monolayer tissue culture cells. Because the $\alpha$ chains of luteinizing hormone and HCG are virtually identical in their amino acid sequence, the $\beta$ chain of HCG was chosen as antigen. Purified $\beta$ chain of HCG (supplied by Dr. Vernon Stevens, Ohio State University School of Medicine, Columbus, Ohio) was dissolved in phosphate-buffered saline (100 µg $\beta$ chain in 0.5 ml of 0.5 M phosphate-buffered saline, pH 7.4), emulsified with complete Freund’s adjuvant, and injected directly 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 (24) containing HCG (Organon, Inc., West Orange, N. J.) to obtain the specific anti-$\beta$-chain antibody. As has been shown previously (24), antibodies obtained by this procedure are electrophoretically pure and consistently show insignificant nonspecificity. The sheep anti-rabbit $\gamma$-globulin antiserum was similarly purified using an acrylamide affinity column containing purified rabbit $\gamma$-globulin. The specific sheep antibodies were then conjugated (28) 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 serum were absorbed with human spleen powder to reduce nonspecific adsorption to test tissues. In all sections of tumors examined, normal tissue surrounding the tumor was included as an internal control.

Human placenta (8 weeks to term) and BeWo cells in tissue culture (established from trophoblastic tumor cells of a postgestational human choriocarcinoma that synthesizes HCG (33) supplied by Dr. Roland Patillo, Marquette School of Medicine, Milwaukee, Wis.) were used as positive controls. BeWo cells were grown in Eagle’s minimal essential media supplemented with 10% fetal calf serum. The cultures were trypsinized and placed in chambered slides 48 hr before use.

Human malignant tumors were collected in the operating room or at necropsy, embedded in Aims O.C.T. (Aims Co., Miles Laboratories, Elkhart, Ind.), quickly frozen in dry ice and ethanol, and stored at −20° until used.

Results

The normal rabbit serum controls of placenta, BeWo cells, and all other tissues were consistently negative. In addition, when reacted with the anti-$\beta$-chain antibody, normal tissues surrounding the tumors were consistently negative. These normal tissues included lung, liver, kidney, uterus, adipose tissue, lymph nodes, spleen, and urinary bladder. In placenta, the anti-$\beta$-chain antibody was localized in the cytoplasm of syncytiotrophoblastic cells. In addition, a discrete dark brown reaction product was present at the outer surface of the chorionic villi. When reacted with...
the anti-β-chain antibody the BeWo cells contained discrete positive cytoplasmic granules (Fig. 1) which were especially prominent in the larger multinucleated cells in the cultures.

The small mononucleated cells, the counterpart of normal cytotrophoblast, were negative. Material cross-reacting with the anti-β-chain antibody was present in the malignant cells of 25 human tumors (Table 1). The reaction product in the malignant cells was localized in the cytoplasm (Figs. 2 and 3), nuclei were consistently negative and, occasionally, a discrete band of staining at or near the surface of the malignant cells was prominent. The percentage of cells positive in a given tumor varied considerably. For example, in some tumors, as many as 90 to 95% of the malignant cells examined were positive; in other tumors, only 10 to 15% of the cells contained reaction product. In general, it appeared that the most anaplastic or poorly differentiated cells tended to be positive, whereas well-differentiated cells in general showed a tendency to be negative. Both stroma and normal tissues present in the sections were negative. Only 3 tumors were negative; all were adenocarcinomas (Table 1). Whether these 3 tumors are really negative or whether they are the result of a very small sample or of extensive necrosis resulting in nonspecific adsorption of the antibody is not clear at this time.

**Discussion**

An increasing number of observations regarding the resemblance of neoplasms to embryonic tissues have been made, including immunological tolerance by the host, invasion into adjacent tissues, and production of similar biochemical markers. More recently, emphasis has been focused on the production of these similar antigenic markers, since it was noted that, during oncogenesis, fetal genes are expressed, _i.e._, carcinomembryonic antigen (16), pancreatic embryonic antigen (3), and α-fetoprotein (1). Such expression of fetal genes by tumors may also result in the production of HCG, one of the earliest embryonic genes to be expressed in ontogeny (19).

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, 5, 11, 18, 21, 23, 26), it suggests that this substance could be partially responsible for nonrejection. However, the concentration of HCG required to produce complete inhibition of the T-cell response has been reported (7, 14) and is currently under evaluation as a diagnostic test for neoplasia (37). The present work localizing the β 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 embryonic tissue should be produced by such a heterogeneous group of tumors.

We would like to postulate that a parallelism may exist 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 (20). Both placental and neoplastic tissues produce a T-cell-inhibiting substance resembling HCG, which may react with T-cell to prevent maturation of the immune response (Chart 1). In another animal system, it has been suggested that an immunosuppressive molecule exists in the mouse that is produced by both trophoblast and malignant cells (13). Interestingly, an inversion in the levels of T- and B-cells in early human pregnancy has recently been reported (35), and the inversion parallels the rise and fall of serum HCG levels. That is, the level of T-cells was the lowest when the concentration of HCG was the highest.

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**Table 1**

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma, prostate</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, pancreas</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, colon</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, lung</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma, stomach</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma, breast</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Squamous cell carcinoma, cervix</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma, lung</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Transitional cell carcinoma, kidney</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Seminoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dysgerminoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>3</td>
</tr>
</tbody>
</table>

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. Previous reports (6, 34) implicating cell surface mucoprotein complexes as immunoprotective shields in both normal and malignant trophoblast would also agree with this hypothesis.

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 carboxy-terminal end of the β chain (9). The structure of this “tail” region of the β chain of HCG, which is absent from the β chain of luteinizing hormone, is reminiscent of collagen and includes several prolines and 5 serine-linked carbohydrates, 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 (7, 14) and is currently under evaluation as a diagnostic test for neoplasia (37). The present work localizing the β 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 embryonic tissue should be produced by such a heterogeneous group of tumors.

We would like to postulate that a parallelism may exist 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 (20). Both placental and neoplastic tissues produce a T-cell-inhibiting substance resembling HCG, which may react with T-cell to prevent maturation of the immune response (Chart 1). In another animal system, it has been suggested that an immunosuppressive molecule exists in the mouse that is produced by both trophoblast and malignant cells (13). Interestingly, an inversion in the levels of T- and B-cells in early human pregnancy has recently been reported (35), and the inversion parallels the rise and fall of serum HCG levels. That is, the level of T-cells was the lowest when the concentration of HCG was the highest.
The unique surface distribution of this molecule on the placental surface interacts with a T-cell-inhibitory substance that prevents further maturation of the immune response. CO: maternal HLA; •, A: paternal HLA.

This proposed mechanism could account for both the specific immunosuppression in the mother during pregnancy and the recovery of her immunocompetence during the postnatal period. Such specific immunosuppression has been reported previously (10, 32). Those experiments showed that maternal lymphocytes that failed to respond with blastogenic transformation in mixed lymphocyte culture to the lymphocytes of their newborn sons had intermediate responses to the lymphocytes of their spouses, whereas they responded normally to the lymphocytes of another male. A similar mechanism might be operative if HCG is present on tumor cell surfaces specifically suppressing those T-cells capable of reacting with tumor antigens. 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.

References
Fig. 1. Tissue culture of BeWo cells (48-hr subculture). a, phase-contrast micrograph of the same area as b. b, anti-β-chain HCG antibody followed by peroxidase-labeled sheep anti-rabbit antibody. The dense deposits of reaction product are present in discrete cytoplasmic granules in the giant cell at the left upper corner. Original magnification, ×220.

Fig. 2. Six-μm frozen section of breast infiltrating ductal carcinoma. Group of malignant cells surrounded by dense stromal reaction. a, H & E. b, anti-β-chain HCG 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. Original magnification, ×100.
Fig. 3. Rhabdomyosarcoma, conditions as for Fig. 2. a, H & E; b, Anti-β-chain antibody. Original magnification, × 100. c, Anti-β-chain antibody. Original magnification, × 400. Many tumor cells give a positive reaction, particularly the large pleomorphic cells.
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