Serotonin and Histamine Production by Human Carcinoid Cells in Culture

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ABSTRACT

We are reporting on the first human carcinoid cells ever cultured in vitro. These cells, termed CGP, originated from a jejunal carcinoid tumor. Before tumor resection, the 29-year-old male patient presented high levels of blood serotonin and histamine and also of urinary serotonin and 5-hydroxyindole-acetic acid; values returned to normal 9 days after resection. CGP cells exhibit a very slow multiplication rate; generation time is about 10 days. From the first subculture, the main cytological, ultrastructural, and biochemical features of CGP cultures remain unchanged. The cells show most of the enterochromaffin cell histomorphological characteristics; for example, cytoplasmic granulations, specific of argyrophilic cells, can be seen both by electron microscopy and by light microscopy (preceded by silver impregnation). The high amounts of serotonin and histamine found by highly specific radioenzymatic assays in the supernatant of CGP cultures indicate that, after 6 months (25 subcultures), CGP cells have retained the main metabolic characteristics of the original tumor, i.e., the ability to synthesize, store, and release both serotonin and histamine.

INTRODUCTION

The ubiquity of 5-HT and HA has generated a great deal of speculation concerning the possible participation of these amines in health and disease (1-3). In contrast to the very large number of studies concerning 5-HT and HA in the central nervous system, few studies exist concerning these amines in the gastrointestinal tract, HA is thought to be present in "non-mast cell" stores, the nature of which is unknown (1). Bowel 5-HT is primarily located within EC and EC-like cells. These cells belong to the amine precursor uptake decarboxylase system which also produces low-molecular-weight polypeptide hormones. It is not clear whether 5-HT in these cells functions as a hormone in its own right or whether it is in some way necessary for the synthesis, storage, or release of the polypeptide hormones (3).

Carcinoid tumors are a proliferation of EC or EC-like cells. The symptoms, clinical course, and physical and laboratory findings are so variable that, even today, histological criteria for the diagnosis of carcinoidosis was supported by classical histomorphological examination (8) of the tumor.

5-HIAA, 5-HT, and HA Measurements. A high-pressure liquid chromatography-fluorimetric assay (5) was used to determine 5-HIAA levels. Highly specific radioenzymatic assays were used to measure HA (13) and 5-HT (12, 14). In brief, they consisted of the transfer of a "H-CH3 group from S-[3H]adenosylmethionine to HA or 5-HT (the latter previously converted to N-acetylserotonin by treatment with acetic anhydride). The partially purified enzymes used to mediate the transfer of the "H-CH3 group were (a) histamine N-methyl transferases from rat kidney for HA and (b) hydroxyindole-O-methyl transferase from beef pineal for 5-HT. The products of this reaction, N-[3H]methylhistamine and [3H]melatonin, respectively, were freed from excess S-[3H]adenosylmethionine by alkaline chloroform extraction and thin-layer chromatography.

Light and Electron Microscopy. For light microscopy, cells were stained with hematoxylin-eosin or submitted to silver impregnation according to Fontana's method (8). For electron microscopy, cells were fixed in glutaraldehyde and postfixed in osmium tetroxide. Thin sections of the dehydrated cells, embedded in Epon M2, were stained with uranyl acetate and lead citrate. Electron micrographs were taken on a Philips 301 electron microscope.

RESULTS

Culture of Human Carcinoid Cells. Less than 1 hr after surgical excision, the tumor, cut into small pieces, was submerged twice in 0.25% and once in 0.60% trypsin in phosphate-buffered saline for 20 to 30 min each time at room temperature. After each trypsin treatment, supernatants were filtered through sterile gauze and centrifuged at low speed. The cell pellets were pooled and washed with phosphate-buffered saline and Dulbecco's medium. Cells were then optically counted in a Nageotte hemocytometer after addition of trypan blue, suspended (5 x 10^5 cells/ml) in Dulbecco's medium containing antibiotics (50 IU of penicillin per ml and 100 µg of streptomycin per ml) and 20% fetal calf serum, seeded in culture-adapted plastic flasks and incubated at 37 °C in a 5% CO2-enriched atmosphere. Two days later, no cell adhesion had occurred, but cells still looked alive and were unstainable by trypan blue. Then, one-half of the cell suspen-
sions were transferred to new sterile plastic flasks. The residual cells were refed with fresh medium. This procedure was repeated twice with a 3-day interval. The first adherent cells forming an irregular, subcultivable cell monolayer were observed between 8 and 14 days after plating. These cells, which we term CGP, exhibit a very slow multiplication rate with a generation time of about 10 days. They have been subcultured 25 times for a period of time exceeding 300 days.

**Characterization of Human Carcinoid Cultured Cells.** From the first subculture, the main cytological, ultrastructural, and biochemical characteristics of CGP cells remain unchanged. They are as follows.

As observed by light microscopy (hematoxylin-eosin staining) cells are preferentially arranged in a cord-like pattern. They exhibit a pyramidal or oval shape with a common nucleo-plasmatic ratio (Fig. 1a). After silver impregnation, numerous granulations, characteristic of argyrophilic cells, can be seen in the cytoplasm (Fig. 1b).

Electron microscopic studies of CGP cells show numerous microvilli of irregular length extending along the cytoplasmic membrane (Fig. 2, a and b) and small vacuoles reminiscent of pinocytic vesicles because of their size and location. CGP cytoplasm also contains numerous larger empty vacuoles (Fig. 2, c and d) occasionally accompanied by myelin bodies (Fig. 2c). Pleomorphic granulations (oval or round shapes, 200 to 600 nm in diameter) are also present to a moderate extent (Fig. 2, c and d). This granular material, which seems to be encircled by a single envelope membrane, shows a variable electron density.

Chart 1 presents the results obtained concerning the detection of 5-HT and HA in the supernatant fluids of CGP cells. It can be seen that high amounts of both substances are detectable at each passage level at which they were studied. On the contrary, supernatant fluids of several other human cell lines (HeLa, WI38, and AV3) always gave negative results.

**DISCUSSION**

The results presented here indicate that human carcinoid cells can be cultured in vitro under long-term conditions and that the cultured cells can retain their main characteristics of metabolic differentiation, i.e., to synthesize, store, and release both 5-HT and HA. Two aspects of CGP cells should be emphasized: (a) they have a very slow multiplication rate which seems to correlate with the slow growth of carcinoid tumors (3, 4, 10); and (b) relatively few granules with the morphology characteristic of amine precursor uptake decarboxylase tumor granulations present in the cytoplasm are detectable by electron microscopy. Since CGP cells are derived from a carcinoid jejunal tumor, one would in fact expect to find a higher quantity of this type of granule indicative of the cell origin. However, the larger vacuoles seen in electron microscopy (Fig. 2, c and d) may correspond to granules the content of which has not been secreted. This explanation is favored by the fact that the 5-HT content of CGP cells is low compared to the extracellular level (0.1 versus 1.5 µmol). In addition, a greatly elevated number of filled granules is observed in CGP cytoplasm using fluorescence microscopy after loading the cells with mepacrine, a specific marker of 5-HT organelles in blood platelets (9).³

³ H. P. Lorez, Hoffman La Roche Inc., Basel, Switzerland, personal communication.

In conclusion, we hope that this first in vitro culture of human carcinoid cells, showing most of the EC cell histomorphological characteristics and producing both 5-HT and HA, will make possible future studies concerning cellular transport, storage, and secretion of the hormones produced by carcinoid tumors (11), pharmacological inhibition of 5-HT and HA secretion, and other aspects of 5-HT and HA biochemistry and physiology (14).

**ACKNOWLEDGMENTS**

The excellent technical assistance of D. Pasques is gratefully acknowledged for histamine and 5-HT determinations. We also wish to thank Professor P. Clot for providing the carcinoid tumor and Dr. M. Da Prada, Pharmacological Research Department, Hoffmann La-Roche Inc., Basel, Switzerland, for the critical reading of the manuscript.

**REFERENCES**

Human Carcinoid Cell Culture


Fig. 1. Light micrograph of CGP cells. In a, with hematoxylin-eosin staining, cells appear rather fibroblastic (long cells with a large cytoplasm), but they are randomly distributed and never give a regular cell monolayer as fibroblast cultures usually do. X 400. In b, silver impregnation clearly shows the presence of granules in the cytoplasm of CGP indicating the argyrophilic pattern of these cultured cells X 400.
Fig. 2. Electron micrographs of CGP cells. a, general aspect of CGP cells. ×2,500. In b, the membrane has microvilli with empty vacuoles subjacent to it, suggestive of pinocytosis. ×19,000. In c, few mitochondria, granular secretions, regular ergastoplasm, and myelin figures are also encountered. ×7,000. Inset, details of secretory granule. Bar, 600 nm. d, partial view of the cytoplasm showing numerous empty vacuoles and considerable amount of granular secretion (see text). ×5,000.
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