PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 176, 503-507 (1984)

RAPID COMMUNICATIONS

ISOLATION AND CULTURE OF HUMAN INTESTINAL SMOOTH MUSCLE CELLS

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Abstract. Intestinal smooth muscle cells were isolated from human bowel and maintained in culture through several passages. These cells were obtained by enzyme digestion of slices taken from the circular layer of the muscularis propria of human jejunum. When subcultured, they initially flattened out and then began proliferating after 3 days. After 3 weeks in culture, they began aggregating into ridges. Fluorohistochemical staining revealed numerous prominent actin stress fibers. When these cells were exposed to the C-terminal octapeptide of cholecystokinin they contracted in a dose-dependent fashion. The availability of human intestinal smooth muscle cells in culture will considerably enhance our ability to study the contractile, proliferative and connective tissue responses of the smooth muscle of the human gastrointestinal tract.

Intestinal smooth muscle cells play a critical role in the motility and physical integrity of the gastrointestinal tract. Contraction and relaxation of the deep muscle layer, the muscularis propria, results in normal aboral propulsion and mixing of luminal contents. The role of the superficial layer, the muscularis mucosae, is unclear but may involve motility of villi (1). Destruction of the muscularis propria as seen in the toxic megacolon complicating ulcerative colitis, leads to decreased motility, progressive dilatation and perforation of the bowel (2). Chronic inflammation of the bowel wall leads to thickening of the muscle coat, collagen deposition and narrowing of the gut lumen (3). Until now, sterile cultures of smooth muscle cells from human bowel have not been available. We describe here the techniques we have developed for the isolation and culture of these cells. Their availability in long term culture should considerably enhance our ability to study the physiology and pathology of the smooth muscle of the human gut.

Materials and Methods.

Sources of Tissue. Normal jejunal tissue was obtained from patients undergoing gastric bypass operations. Tissue from the jejunum was used preferentially because it is relatively free of gastrointestinal microorganisms.

Tissue Processing. Once resected, the tissue was immediately placed in sterile phosphate-buffered saline ($4^{o}C$) and transported to the tissue culture facility. Under sterile conditions, mucosa was separated from muscularis by blunt dissection. Slices were taken from the center of the circular layer of the muscularis propria with a Stadie-Riggs tissue slicer, minced and incubated overnight in Dulbecco's Modified Eagle's Medium (DMEM) containing 1% crude collagenase (Grade CLS11, Worthington Diagnostic Systems, Inc.). The following day, released cells were separated from tissue debris by low speed centrifugation (40 x g), washed several times and 2 x 10^6 cells were placed in 100 mm culture plates with 10 ml DMEM containing fetal bovine

0037-9727/84 $1.50

serum (FBS) (GIBCO) and 25 mM Tricine (pH 7.4). Culture medium was replaced every third day. When the smooth muscle cells became confluent after 3 weeks in culture, they were released from the plates by trypsinisation (0.1%) and replated at 2 x 10^6 cells per 100 mm plate. The smooth muscle cells were observed daily and photographed for a record of morphologic changes.

Fluorohistochemistry. Cells were freed from the culture plate surface by trypsinisation. A drop of culture medium (20 ul) containing 5 x 10^4 cells was applied to a sterile glass coverslip (22 x 22 mm) in a 100 mm petri dish, and the cells were allowed to attach for 30 minutes at 37°C in a moist atmosphere. An additional 6 ml of DMEM, 10% serum, was slowly added to the culture dish and this was again placed in a 37°C moist atmosphere incubator. At 24 and 48 hours, coverslips were rinsed in phosphate buffered saline (pH 7.6, PBS), and they were placed in 4% paraformaldehyde-PBS for five minutes at room temperature. The fixed slides were washed for five minutes three times in PBS and they were then permeabilized in PBS containing 0.1% Triton X-100 for five minutes. The Triton X-100 treated coverslips were washed three times in PBS as described above. Rhodamine-phalloidin (Microbiological Probes, Inc., Junction City, Oregon) was layered onto the top of the coverslips at 1:1000 dilution and allowed to incubate at room temperature for 30 minutes. The coverslips were washed with PBS three times and then mounted in glycerol:PBS (9:1) on a glass microscopic slide. The stained coverslips were viewed in a Zeiss IM 35 inverted microscope with fitted Rhodamine filters and epifluoresence. Cellular stress fiber patterns were recorded with Ektachrome 400 color 35 mm film uprated to 1600 ASA.

Measurement of Contractile Response. The procedure as described by Bitar, et al. (4) was used to measure the contractile response of the human intestinal smooth muscle cells to CCK-octapeptide. A solution containing various concentrations of the C-terminal octapeptide of cholecystokinin (CCK-OP, Squibb Institute for Medical Research, Princeton, NJ) was added to the 6 day old cultures of human intestinal smooth muscle cells attached to coverslips. The reaction was stopped after 30 seconds by the addition of acrolein to give a final concentration of 1.0%. Cell length was measured by image-splitting micrometry and for each test, the length of 50 cells in successive random microscopic fields was measured and the mean length calculated. Contraction was expressed as the percentage decrease in mean cell length from control. A dose response curve was constructed for CCK-OP at concentrations of 10^{-9} and 10^{-11}M (3 experiments); 10^{-10} and 10^{-13}M (2 experiments) and 10^{-14}M (1 experiment).

Growth Curve. Smooth muscle cells released from the bottom of culture plates by trypsinisation were plated into multiwell culture plates (Costar #3524, Cambridge, MA) at two densities; 10^4 and 10^5 cells per well. 1 ml DMEM containing 10% FBS was added to each well. Cells were released from the well bottoms with trypsin at daily intervals and the total number of cells in each of 6 wells were counted using a Coulter counter (Coulter Electronics, Automatic Blood Cell Counter, Model D2N).

Results. Collagenase digestion of approximately 10 cm^2 of muscularis tissue yielded approximately 1 x 10^7 viable cells. Viability by trypan blue exclusion was greater than 60%. Newly released, oblong-shaped cells (primary culture) attached to the plate within 12 hours and by 7 days they had flattened out. Following treatment with trypsin and subculture, cells flattened out within 24 hours and began proliferating after 2-3 days in culture. These cells demonstrated prominent intracellular fiber-like structures on light microscopy. Fluorohistochemical staining with Rhodamine-phalloidin 48 hours after passage confirmed the presence of prominent actin stress fibers (Fig. 1). (Phalloidin, a phallotoxin, has been demonstrated to bind specifically to F-actin (5)). After 3 weeks in culture, cells began to aggregate forming ridges (Fig. 2).

The contractile response (expressed as percent decrease in cell length) of the human intestinal smooth muscle cells to CCK-OP was dose-dependent (Fig. 3).

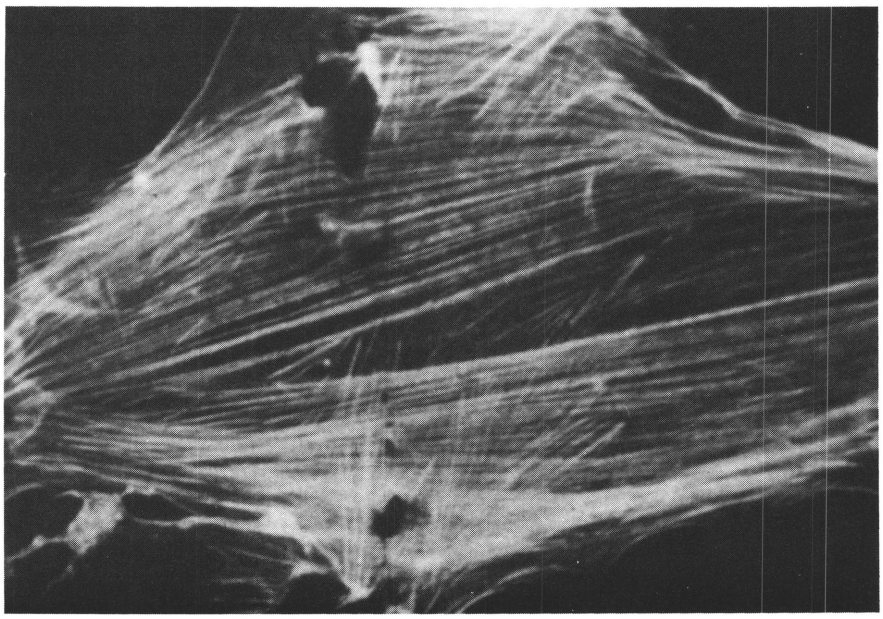

Figure 1

Human intestinal smooth muscle cells stained with Rhodamine-phalloidin demonstrating numerous, dense actin stress fibers (280 x). Cells were passaged 48 hours prior to staining and were viewed with epifluorescence.

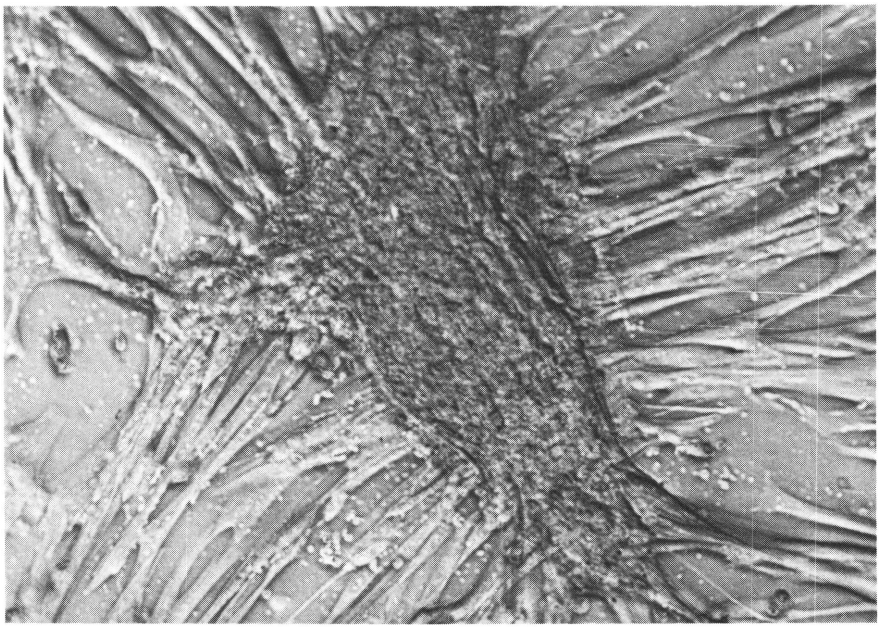

Figure 2

Cells forming ridges after 3 weeks in culture (70 x).

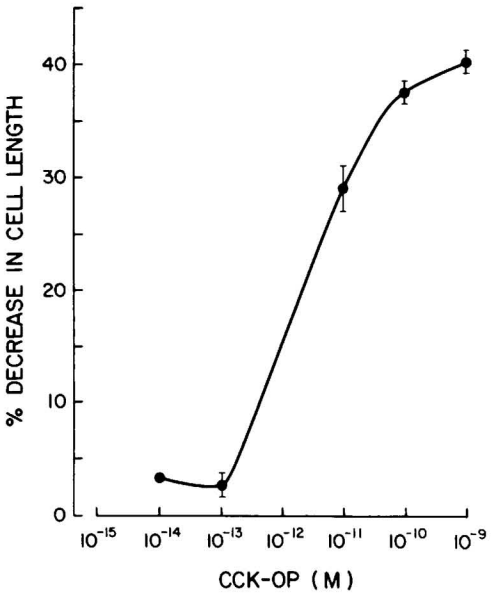

Figure 3

Dose-response curve for the effect of CCK-OP on cultured human intestinal smooth muscle cell contraction. Data are average values and the bars indicate the range of values.

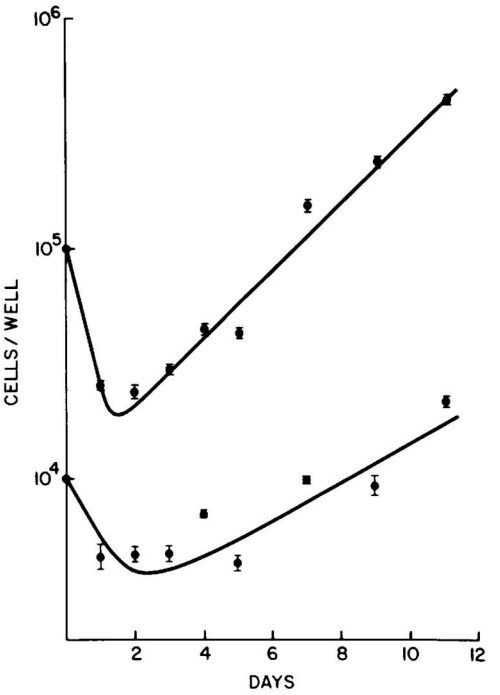

Figure 4

Growth curves of human intestinal smooth muscle cells plated at densities of 10^4 and 10^5 cells/1 ml well. Cells were plated in Costar multiwell plates and were counted in triplicate following trypsinisation.

When the smooth muscle cells were subcultured at 10^4 and 10^5 cells per culture well, there was an initial 2 to 3 day lag in proliferation (Fig. 4). This lag period was followed by an 8 day period of linear proliferation without a density dependent inhibition of growth.

Discussion. Techniques for the isolation of smooth muscle cells have been available for some time (6). However, sterile isolation and long term culture of smooth muscle cells from human intestinal tissue has not yet been previously reported. The technique we have described provides a ready source of a large number of human cells, the availability of which will facilitate studies of the contraction, proliferation and connective tissue synthesis of human intestinal smooth muscle. The proliferative response of these cells to fetal bovine serum simulates that of other non-human smooth muscle cells in culture (6,7). Proliferation while in a "modulated" state, the return to a contractile state following confluence and the subsequent formation of aggregates or ridges, have been well described for smooth muscle cells from other sources (6). The very prominent actin filaments seen by fluorohistochemistry are characteristic of smooth muscle cells and are not normally observed in fibroblasts or endothelial cells (7). In addition, these cells contracted in a concentration-dependent fashion to CCK-octapeptide. The concentrations of CCK-OP required for half maximal (D_{50}= 10^{-11} to 10^{-12}M) and maximal contractile response (40.3% decrease in cell length at 10^{-9}M) of these cultured human jejunal smooth muscle cells were similar to those reported for freshly isolated, dispersed human antral smooth muscle cells (4). These findings substantiate the identity of these cultured human intestinal cells as smooth muscle cells. Studies utilizing these cells in long term culture will certainly enhance our

understanding of the contractile and connective tissue responses of the human gut under a variety of both physiologic and pathologic conditions.

This work was supported by a grant from the National Foundation for Ileitis and Colitis. The authors would like to thank Dr. Harvey Sugerman for his encouragement and assistance, Kristi Eastburn for technical assistance and Nonie Barnstein for help in preparing the manuscript.

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Received April 23, 1984.
P.S.E.B.M. 1984, Vol. 176.
Accepted June 20, 1984.