Eur J Cancer, Vol. 27, No. 5, pp. 599–604, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00


# Vectorial Release of Carcinoembryonic Antigen Induced by IFN-γ in Human Colon Cancer Cells Cultured in Serum-free Medium

Stephen Baghdiguian, Bernard Verrier, Monique Roccabianca, Gilbert Pommier, Jacques Marvaldi and Jacques Fantini

**Confluent monolayers of intestinal cell lines are useful models for studies of intestinal epithelial structure and function. Three cell lines have retained morphological and functional properties of intestinal epithelial cells compatible with such studies: Caco-2, T84 and HT29. However, the requirement of fetal bovine serum for the culture of these cells does not facilitate the design of experiments dealing with growth factors or hormonal regulation. The clonal intestinal cell line HT29-D4 can be cultured as fully differentiated epithelial monolayers in a synthetic medium containing transferrin, selenous acid, epidermal growth factor and suramin, a potent differentiation inducer. In the present study it is shown that HT29-D4 cells grown on permeable substratum in this synthetic medium developed electrically active monolayers consisting of columnar cells with morphological characteristics of normal enterocytes. After metabolic labelling with [$^{35}$S]-methionine, HT29-D4 monolayers released most of their radiolabelled secretory proteins preferentially in the basal compartment of the cell culture chamber. However, the carcinoembryonic antigen, shown to be present in the apical plasma membrane, was exclusively released apically. This oriented release was stimulated by recombinant gamma-interferon (IFN-γ) added only in the basal chamber, suggesting a basolateral restriction for IFN-γ receptors.**
*Eur J Cancer*, Vol. 27, No. 5, pp. 599–604, 1991

## INTRODUCTION

THE INTESTINAL surface epithelium is mainly composed of two polarised cell types, namely goblet and absorptive cells [1, 2]. Goblet cells are specialised in the biosynthesis, storing and secretion of mucus granules. Absorptive cells are responsible for the digestion of specific nutrients and ion and water transport. The difficulty to establish confluent monolyers from intestinal primary cultures [3] has compelled researchers to use tumoral intestinal cell lines as models for the study of intestinal functions. Among these cell lines, three have retained structural and functional properties of individual species of intestinal epithelial cells: Caco-2, T84 and HT29. Caco-2 [4, 5] and T84 [6, 7] cells differentiated at confluency into absorptive-like cells showing morphological and transport properties of intestinal crypt cells. Differentiated subclones derived from the HT29 cell line have been obtained after butyrate [8] or polyethylene glycol treatment [9]; some of these clones behaved like absorptive cells while others secreted mucus.

In our laboratory, we have isolated a clone (HT29-D4) which can be induced to differentiate into enterocyte-like cells after glucose starvation [10] or suramin treatment [11]. Suramin is an anticancer drug which prevents the binding of several tumour growth factors to their cell surface receptors [12, 13], thus inhibiting cellular growth [13]. In a previous work [11], we hypothesised that the action of suramin on HT29-D4 cells was mediated by the blocking of a growth stimulatory/differentiation inhibitory autocrine loop, as recently demonstrated in granulosa cells [14].

Because the presence of fetal bovine serum in the culture medium does not allow one to control the level of hormones or other substances under experimental conditions, we have developed a serum-free defined medium able to promote growth and differentiation of HT29-D4 cells. This medium contains EGF, transferrin, selenous acid and suramin [15]. When grown with this synthetic medium in Transwell chambers, HT29-D4 cells form electrically active monolayers. The drug was found to be essential in the defined medium to allow the establishment of electrically active monolayers. In the present work we studied the ultrastructure of these monolayers. Moreover, we demonstrated that the cells grown in defined medium release CEA in the apical compartment of the culture chamber and that this polarised process is stimulated by basal gamma-interferon (IFN-γ).

Correspondence to J. Fantini.
S. Baghdiguian and B. Verrier are at INSERM Unité 270, Faculté de Médecine Nord; M. Roccabianca and J. Marvaldi are at the Institut de Chimie Biologique, CNRS URA 202, Université de Provence; G. Pommier is at the Faculté de Médecine de la Timone, Marseille; and J. Fantini is at INSERM Unité 322, Campus Universitaire de Luminy, BP 33, 13273 Marseille cedex 9, France.
Revised 11 Dec. 1990; accepted 8 Feb. 1991.

## MATERIALS AND METHODS

### *Materials*

Suramin was obtained from Specia (Paris) and prepared as a sterile solution of 100 mg/ml in distilled water and stored at −20°C. The anti-carcinoembryonic antigen (CEA) mouse IgG2a monoclonal antibody MAC 601 (Biosys, Compiegne, France) is directed against epitope 4930 on the human CEA. Culture media and supplements were from Eurobio (Paris). Type I collagen was purchased from Seromed (Berlin). Transwell™ chambers (ref. 3414) were from Costar (Bedford, Massachusetts). Recombinant IFN-γ was a generous gift of Dr P. Milhaud (Montpellier).

*Cell culture*

The human colon adenocarcinoma cell clone HT29-D4 [10] was routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing 25 mmol/l glucose and 10% fetal calf serum (FCS). For cell culture on permeable substratum, HT29-D4 cells were seeded on Nuclepore filters (pore size 3 μm) in 24.5 mm Transwell chambers. The filters were covered with type I collagen as previously described [16]. Cells were seeded at a density of $2 \times 10^5$ cells per $cm^2$ and grown in a synthetic medium consisting of a 1/1 mixture of DMEM and Ham's F12 medium, 15 mol/l "Hepes" pH 7.3, 10 ng/ml selenous acid, 10 μg/ml transferrin, 50 ng/ml epidermal growth factor (EGF) and 100 μg/ml suramin. The medium was changed daily. The transepithelial resistance of each filter was tested before use, to ensure integrity of the monolayer, using a modified Ussing chamber as previously described [15]. Only filters with a resistance greater than 200 Ω $cm^2$ were used.

*Morphological techniques*

Cells were fixed *in situ* with 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer pH 7.3 for 1 h, washed for 10 min in the same buffer containing 6.84% saccharose, postfixed in 1% osmium tetroxide, then dehydrated in graded alcohols and embedded in Epon. Sections were cut perpendicularly to the plane of the cell layer and observed with a Jeol 100C electron microscope.

*Metabolic labelling*

Transwell chambers with confluent monolayers of HT29-D4 cells (transepithelial resistance above 200 Ω $cm^2$) were rinsed three times in 1.5 ml per side of methionine-free DMEM and left in the third rinse for 15 minutes. The medium was then replaced with methionine-free DMEM containing 100 μCi/ml [$^{35}$S]-methionine for 12 h. The medium was collected separately from each side and cell debris removed by centrifugation for 2 min in a microcentrifuge. Aliquots were submitted to a 5–15% gradient of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography.

*Immunofluorescence studies*

Monolayers were extensively washed in phosphate buffered saline (PBS) and fixed in 2% paraformaldehyde in PBS. Pieces of filter were incubated in PBS, 2% bovine serum albumin (BSA) in the presence of the anti-CEA monoclonal antibody for 2 h. After thoroughly washing in PBS, 2% BSA, the cells were labelled with a fluorescein-conjugated rabbit anti-mouse antibody (Sigma, St Louis). After a thorough washing, the pieces of filter were mounted in PBS/glycerol and observed under an Olympus microscope.

*CEA measurement and immunodetection*

The amount of CEA released in the medium was determined with the Abbott CEA-enzyme immunoassay (EIA) monoclonal one-step kit (Abbott Laboratories, Chicago). In some cases, the medium was also incubated with 10 μg of MAC 601 anti-CEA monoclonal antibody in the presence of protein A-sepharose with overnight shaking. The immunoprecipitated material was diluted in sample buffer and submitted to 10% SDS-PAGE. The gel was transferred onto a Nitroscreen™ membrane (New England Nuclear, Boston) and CEA was immunodetected using the anti-CEA monoclonal antibody MAC 601 and $^{125}$I-protein A as a revealing agent.

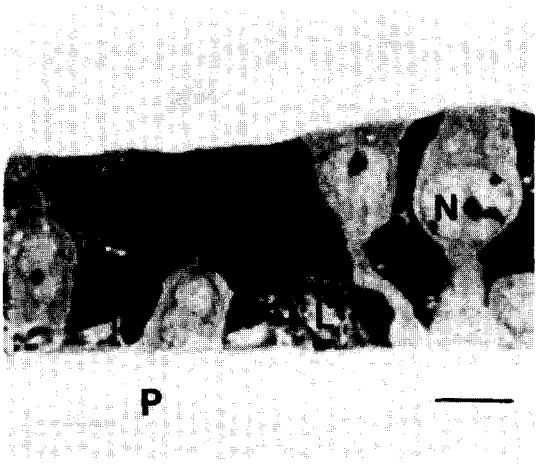


**Fig. 1. Phase-contrast micrograph of HT29-D4 cells seeded on a collagen-coated Nuclepore filter and grown for 10 days in synthetic medium. Note the presence of numerous lipid-like droplets (L) in the basal region of the cytoplasm. N = nucleus, P = pore of the filter, bar = 10 μm.**

## RESULTS

*Structural analysis of HT29-D4 cells grown on permeable substratum*

HT29-D4 cells were grown at confluency in synthetic medium on collagen-coated Nuclepore filters as stated in Materials and Methods. 10 days after seeding, the cells formed a monolayer of high (35–40 μm) columnar cells. Numerous lipid-like droplets were clearly visible underneath the nuclei, especially at the light microscope level (Fig. 1). At the electron microscope level, the cells appeared highly polarised (Fig. 2). The plasma membrane was separated by tight junctions into two distinguishable domains. The apical domain was formed by a well organised brush border with densely packed microvilli showing their characteristic cytoskeletal core. Below the tight junctions, the lateral membrane were widely interdigitated and displayed numerous desmosomes ensuring the cohesion of the epithelial sheet. Glycogen particles and numerous large mitochondria with well developed cristae were distributed in all parts of the cytoplasm.

*Polarised secretion of newly synthesised proteins*

Protein secretion from epithelial cells is frequently polarised [17]. The secretion of newly synthesised proteins was studied in HT29-D4 cells after metabolic labelling with [$^{35}$S]-methionine and analysis by SDS-PAGE followed by autoradiography (Fig. 3). The prominent [$^{35}$S]-methionine-labelled polypeptides were mainly observed in the basal compartment of the culture chambers and represent those secreted from the basolateral membrane. The principally labelled protein secreted for the apical membrane was a 97 kD polypeptide (lanes 3 and 5). The most abundant basolateral product was a 44 kD polypeptide (lanes 2 and 4) totally absent in the medium recovered from the apical compartment (lanes 3 and 5). To exclude the possibility that the release of cellular proteins was due to cell damage or cell death occurring during the period of labelling, we performed two experiments. First, we measured the transepithelial resistance of the monolayers before and after the metabolic labelling, and did not notice any change. Moreover, we prepared a total cell extract after a 30 min pulse-labelling of the monolayers. As shown in lane 1, the main labelled proteins in the extract were clearly different from the proteins released in the medium. From

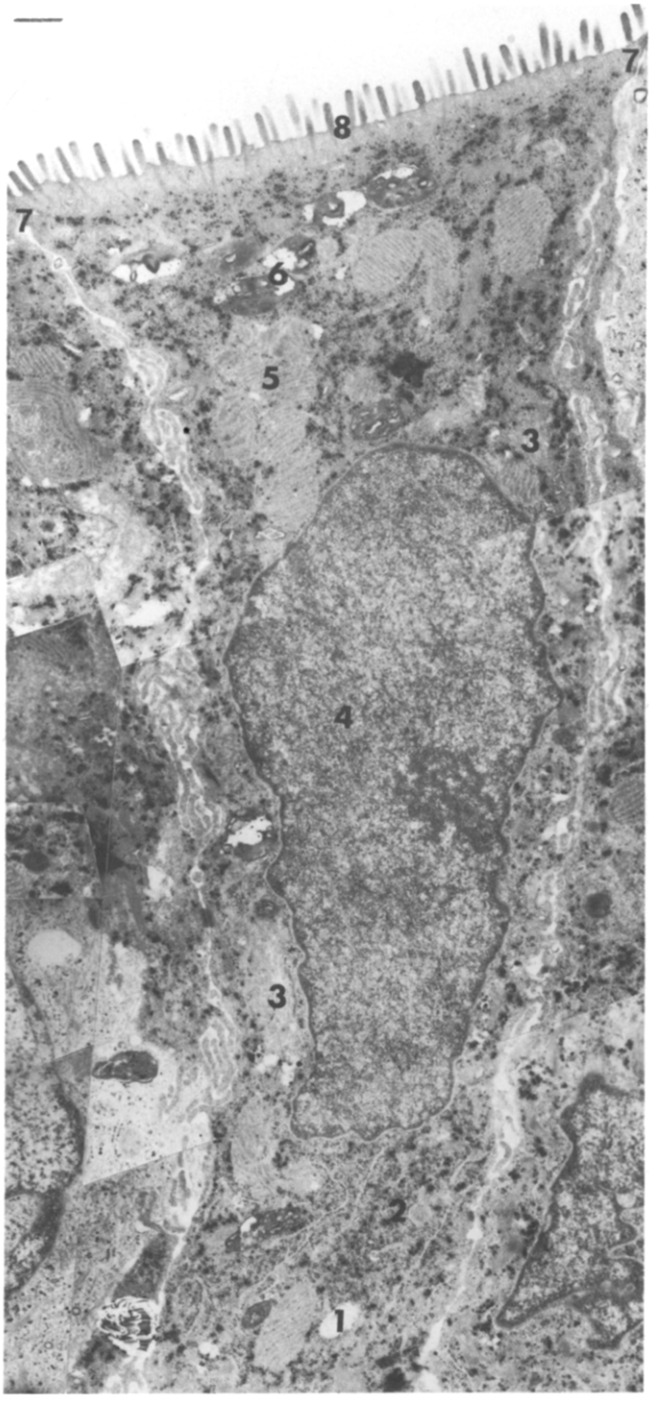


**Fig. 2. Transmission electron micrograph of HT29-D4 cells seeded on a collagen-coated Nuclepore filter and grown in synthetic medium for 10 days. The monolayer is uniformly comprised of highly polarised columnar cells with a basal nucleus and a well-defined brush border. 1 = lipid-like droplet, 2 = glycogen particles, 3 = Golgi apparatus, 4 = nucleus, 5 = mitochondria, 6 = autophagic vacuoles, 7 = tight junction, 8 = brush border. Bar = 1 μm.**

these two observations, we can reasonably conclude that the vectorial release of proteins by HT29-D4 cells is not due to cell damage. Taken together, these results further demonstrate the absolute tightness of HT29-D4 monolayers cultured in synthetic medium, because of their ability to maintain the assymetry of secretion of newly synthesised proteins.

*Effect of IFN-γ on the polarised release of carcinoembryonic antigen (CEA)*

HT29-D4 cells synthesise CEA, which is transported to the brush border and eventually released towards the apical side of the monolayer [18]. The presence of CEA in the brush border of HT29-D4 cells grown in synthetic medium on Nuclepore filters was assessed by indirect immunofluorescence using a highly specific monoclonal antibody. The punctated staining obtained with this antibody was typical of apical labelling and could be assigned to an illumination of microvilli viewed *en face* (Fig. 4). The specificity of the labelling was demonstrated using an irrelevant antibody that gave no staining on the apical side of the cells.

The amount of CEA released by HT29-D4 cells in each compartment of the culture chamber was determined using an immunoenzymatic assay (Fig. 5). CEA was exclusively detected in the medium recovered from the apical compartment, demonstrating its exclusive apical release. Since IFN-γ was recently shown to increase the expression of CEA in human intestinal cells [19], we decided to determine the possible polarisation of this phenomenon. Recombinant IFN-γ was added basolaterally or apically. After a 16 h incubation period, CEA was dosed in both apical and basal compartments of the culture chambers. When IFN-γ (100 U/ml) was added to the apical compartment, only a small increase in the amount of apically-released CEA was observed (125% of the control value). On the contrary, the amount of CEA released in the apical medium was significantly enhanced after basal application of IFN-γ (225% of the control

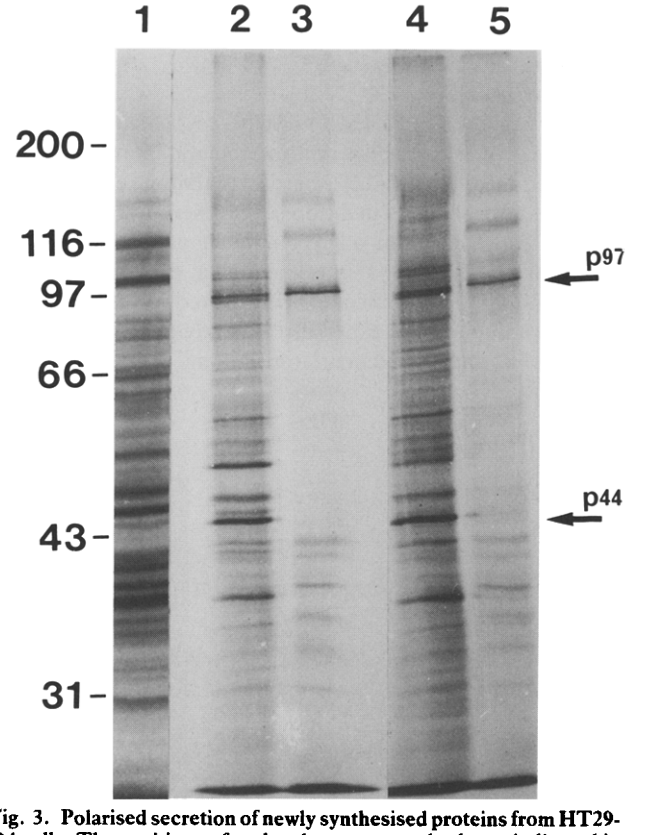


**Fig. 3. Polarised secretion of newly synthesised proteins from HT29-D4 cells. The positions of molecular mass standards are indicated in the margin (in kD). 1: Total cell lysate after solubilisation of the cell monolayer with 1% Triton X 100. These cells were pulsed for 30 minutes with [$^{35}$S]-methionine. 2 and 4: medium collected from the basal compartment (2 separate experiments). 3 and 5: medium collected from apical compartment (2 separate experiments).**

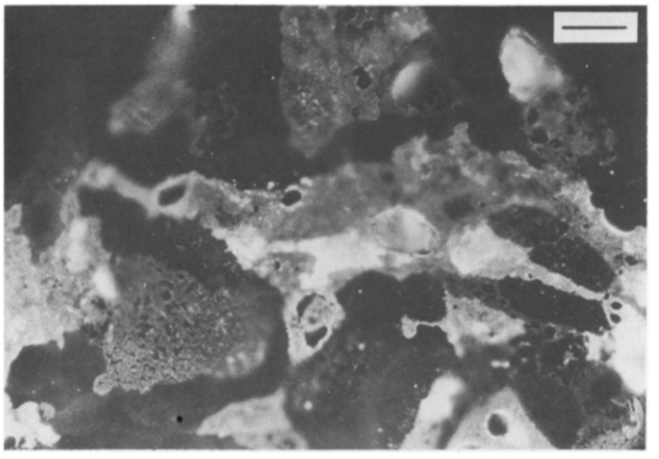

**Fig. 4. Indirect immunofluorescence staining of the apical membrane of HT29-D4 cells with MAC 601 anti-CEA monoclonal antibody. No response was seen when the membrane was stained with an irrelevant antibody. Bar = 25 μm.**

value). To assess that intact CEA molecules were released by HT29-D4 cells under IFN-γ stimulation, the media were submitted to immunoprecipitation with an anti-CEA monoclonal antibody followed by western blotting (Fig. 6). The 180 kD polypeptide corresponding to native CEA molecules was detected only after immunoprecipitation of media collected from the apical compartment (compare lanes 1 with 2, and 3 with 4). Moreover the intensity of the band immunodetected at 180 kD, as analysed by quantitative scanning of the autoradiogram, further demonstrated the higher amount of CEA released in the apical compartment when IFN-γ was applied basolaterally (lane 1) instead of apically (lane 3).

## DISCUSSION

Despite several attempts to culture tumoral intestinal cells in serum-free medium [20–22], the differentiation of these cells has always been obtained in the presence of serum supplemented medium, which contains a great number of substances (e.g. hormones) susceptible to interfere under experimental conditions. Indeed, development of a serum-free synthetic medium is of a great importance, especially for studying the mechanism of action of hormones and neurotransmitters.

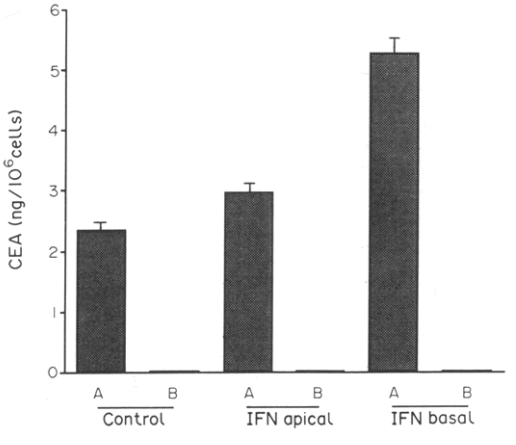


**Fig. 5. Effect of IFN-γ on the amount of CEA released in the culture medium by HT29-D4 cells grown in Transwell chambers. Results are expressed as means (S.D.) of three separate experiments performed in duplicate.**

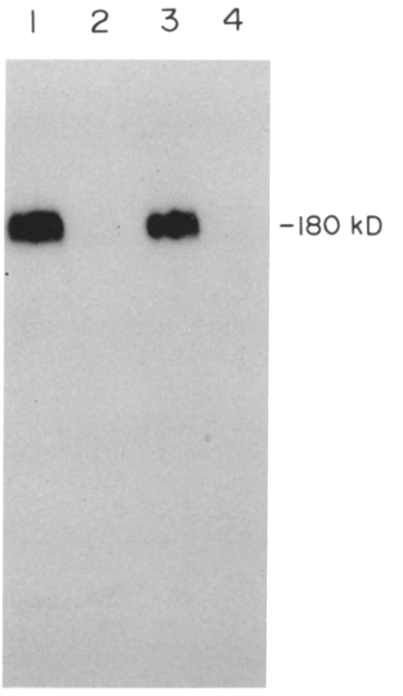


**Fig. 6. Immunoidentification of CEA released by HT29-D4 cells. IFN-γ was applied basolaterally (1, 2) or apically (3, 4) after which apical (1, 3) and basal (2, 4) media were collected and submitted to immunoprecipitation with MAC 601 anti-CEA antibody followed by Western blot. A unique polypeptide migrating at 180 kD, corresponding to native CEA molecules, was recognised by anti-CEA antibodies.**

Recently we reported that suramin, a drug currently used in the therapy of advanced malignancy [23], was able to induce the enterocytic differentiation of HT29-D4 cells [11]. Furthermore, a similar differentation was obtained by adding suramin (100 μg/ml) to a completely defined medium that was enriched in EGF (50 ng/ml) to improve cell growth [15]. HT29-D4 cells grown in this synthetic medium were able to form electrically active monolayers, as assessed by the establishment of a significant transepithelial resistance (250 Ω $cm^2$) [15]. In the first part of this work, we studied the morphology of these cells by electron microscopy. HT29-D4 cells grown in defined medium on Nuclepore filters formed a regular monolayer of columnar cells. Their cytoplasm was rich in large mitochondria and glycogen particles. This is in agreement with our recent multinuclear magnetic resonance spectroscopy study that demonstrated the development of oxidative metabolism and glycogen synthesis during the differentiation process of HT29-D4 cells [24]. The tight junctions conferred a perfect tightness to the monolayer, as demonstrated by the establishment of a stable transepithelial resistance of the same order of the one recorded for Caco-2 cells [5] and HT29-D4 cells grown in serum-containing medium [16]. Moreover, HT29-D4 cells grown in defined medium secreted a variety of newly synthesised products preferentially from their basolateral surface, as already demonstrated for Caco-2 cells [17], thus confirming the tightness of the filter-grown monolayer.

Recently we demonstrated that HT29-D4 cells cultured on permeable filters in the presence of serum-containing medium sorted the carcinoembryonic antigen (CEA) in the apical membrane domain, then released this molecule into the medium bathing the apical side of the monolayer [18]. When grown in suramin-containing defined medium, HT29-D4 cells expressed CEA exclusively on the brush border, as demonstrated by

indirect immunofluorescence staining. These data prompted us to test the action of IFN-γ, which stimulates CEA expression in colon carcinoma cells [19], on the polarised release of CEA. We applied recombinant IFN-γ (100 U/ml) either basolaterally or apically on tight HT29-D4 monolayers cultured in serum-free medium. During the incubation period (16 h), suramin was omitted because of its interference with the CEA release process [25]. We showed that IFN-γ significantly increased the amount of apically-released CEA, providing that it was applied basolaterally. These data support the idea that functional receptors for IFN-γ [26] are present in the basolateral membrane of differentiated HT29-D4 cells.

It should be emphasised that IFN-γ is produced by intraepithelial lymphocytes [27], a category of leucocytes found in the intestinal epithelium in close relation with the base of epithelial cells [28]. Hence, it is tempting to speculate that this lymphokine is able to regulate the release of CEA in the normal intestinal lumina. Moreover, one should note that IFN-γ is also able to enhance the expression of HLA-ABC and HLA-DR antigens [29] and the secretory component (SC) of IgA [30] in HT29 cells. All these molecules (HLA-ABC, HLA-DR, SC and CEA) share a structural homology with the immunoglobulin supergene family products [31–33]. It is likely that IFN-γ produced by intraepithelial lymphocytes regulates the expression of molecules possessing immunoglobulin-like domains in absorptive intestinal cells, and that this regulation is mediated by the interaction of IFN-γ with a specific receptor present on the basolateral surface of enterocytes.

The mechanism of interferon-induced CEA release is still unknown. The CEA molecule is anchored to the cell membrane by a glycophospholipid-containing phosphatidylinositol [34] which can be cleaved from the membrane by a bacterial phosphatidylinositol-phospholipase C [25, 35]. This enzymatic cleavage of CEA could be, at least in part, under the control of IFN-γ.

In conclusion, we report for the first time the culture of epithelial cells of intestinal origin as confluent polarised monolayers under perfectly known conditions. The ultrastructural analysis of these monolayers demonstrated that the cells were highly polarised and displayed most of the morphologial features of normal enterocytes. These cells were also functionally differentiated. For instance, intracellular cyclic adenosine monophosphate (cAMP) content increased by basal stimulation with vasoactive intestinal peptide (VIP) [15], in agreement with the restricted basolateral localisation of VIP receptors in HT29-D4 cells [36] and in normal intestinal cells [37]. CEA was present in the apical membrane and its release was oriented as previously described for HT29-D4 cells cultured in serum-containing medium. Using this unique model we demonstrated that IFN-γ, when added in the basal compartment of the culture chamber, induced a significant increase in the amount of CEA released in the apical compartment, and this suggests a basolateral restriction for IFN-γ receptors. Taken together, these data show that the presence of suramin in the defined medium does not alter the morphological and functional properties of HT29-D4 cells. Moreover this model gave us the opportunity to investigate the mechanism of suramin action on cell differentiation.


1. Trier JS, Madara JL. Functional morphology of the mucosa of the small intestine. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract*, 2nd ed. New-York, Raven, 1987, 1209–1249.
2. Levine DS, Haggitt RC. Normal histology of the colon. *Am J Surg Pathol* 1989, **13**, 966–984.
3. Moyer MP. Culture of human gastrointestinal epithelial cells. *Proc Soc Exp Bio Med* 1983, **174**, 9625–9663.
4. Pinto M, Robine-Leon S, Appay MD, *et al.* Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol Cell* 1983, **47**, 323–330.
5. Grasset E, Pinto M, Dussaulx E, Zweibaum A, Desjeux JF. Epithelial properties of human colonic carcinoma cell line Caco-2: electrical parameters. *Am J Physiol* 1984, **247**, C260–C267.
6. Dharmsathaphorn K, McRoberts J, Mandel KG, Tisdale L, Masui H. A human colonic tumor cell line that maintains vectorial electrolyte transport. *Am J Physiol* 1984, **246**, G204–G208.
7. Madara JL, Stafford J, Dharmsathaphorn K, Carlson S. Structural analysis of a human intestinal epithelial cell line. *Gastroenterology* 1987, **92**, 113–145.
8. Augeron C, Laboisse CL. Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. *Cancer Res* 1984, **44**, 3961–3969.
9. Laboisse CL, Maoret JJ, Triadou N, Augeron C. Restoration by polyethylene glycol of characteristics of intestinal differentiation in subpopulations of the human colonic adenocarcinoma cell line HT29. *Cancer Res* 1988, **48**, 2498–2504.
10. Fantini J, Abadie B, Tirard A, *et al.* Spontaneous and induced dome formation by two clonal cell populations derived from a human adenocarcinoma cell line, HT29. *J Cell Sci* 1986, **83**, 235–249.
11. Fantini J, Rognoni JB, Roccabianca M, Pommier G, Marvaldi J. Suramin inhibits cell growth and glycolytic activity and triggers differentiation of human colic adenocarcinoma cell clone HT29-D4. *J Biol Chem* 1989, **264**, 10282–10286.
12. Hosang M. Suramin binds to platelet-derived growth factor and inhibits its biological activity. *J Cell Biochem* 1985, **29**, 265–273.
13. Coffey Jr RJ, Leof EB, Shipley GD, Moses HL. Suramin inhibition of growth factor receptor binding and mitogenicity in AKR-2B cells. *J Cell Physiol* 1987, **132**, 143–148.
14. Mondschein JS, Canning SF, Millller DQ, Hammond JM. Insulin-like growth factors (IGFs) as autocrine/paracrine regulators of granulosa cell differentiation and growth: studies with a neutralizing monoclonal antibody to IGF-I. *Biol Reprod* 1989, **40**, 79–85.
15. Fantini J, Verrier B, Robert C, *et al.* Suramin-induced differentiation of the human colic adenocarcinoma cell clone HT29-D4 in serum-free medium. *Exp Cell Res* 1990, **189**, 109–117.
16. Fantini J, Verrier B, Marvaldi J, Mauchamp J. *In vitro* differentiated HT29-D4 cell line generates leakproof and electrically active monlayers when cultured in porous-bottom culture dishes. *Biol Cell* 1989, **65**, 163–169.
17. Rindler MJ, Traber MG. A specific sorting signal is not required for the polarized secretion of newly synthesized proteins from cultured intestinal epithelial cells. *J Cell Biol* 1988, **107**, 471–749.
18. Fantini J, Rognoni JB, Culouscou JM, Pommier G, Marvalde J, Tirard A. Induction of polarized apical expression and vectorial release of carcinoembryonic antigen (CEA) during the process of differentiation of HT29-D4 cells. *J Cell Physiol* 1989, **141**, 126–134.
19. Kantor J, Tran R, Greiner J *et al.* Modulation of carcinoembryonic antigen messenger RNA levels in human colon carcinoma cells by recombinant human gamma-interferon. *Cancer Res* 1989, **49**, 2651–2655.
20. Murakami H, Masui H. Hormone control of human colon carcinoma cell growth in serum-free medium. *Proc Natl Acad Sci USA* 1980, 77, 3464–3468.
21. Bellot F, Luis J, El Battari A *et al.* Extracellular material secreted by human colonic adenocarcinoma cell lines promotes spreading in serum-free medium and induces neurite outgrowth of PC-12 cells. *Int J Cancer* 1985, **36**, 609–615.
22. Forgue-Lafitte ME, Coudray AM, Bréant B, Mester J. Proliferation of the human colon carcinoma cell line HT29: autocrine growth and deregulated expression of the c-*myc* oncogene. *Cancer Res* 1989, **49**, 6566–6571.
23. Stein CA, LaRocca RV, Thomas R, McAtee N, Myers CM. Suramin: an anticancer drug with a unique mechanism of action. *J Clin Oncol* 1989, 7, 499–508.
24. Galons JP, Fantini J, Vion-Dury J, Cozzone PJ, Canioni P. Metabolic changes in undifferentiated and differentiated human colon adenocarcinoma cells studied by multinuclear magnetic resonance spectroscopy. *Biochimie* **71**, 1989, 949–961.
25. Fantini J, Rognoni JB, Theveniau M, Pommier G, Marvaldi J. Impaired carcinoembryonic antigen release during the process of

suramin-induced differentation of the human colic adenocarcinoma cell clone HT29-D4. *J Cell Physiol* 1990, **143**, 468–474.
26. Hershey GKK, Schreiber RD. Biosynthetic analysis of the human interferon-gamma receptor. Identification of N-linked glycosylation intermediates. *J Biol Chem* 1989, **264**, 11981–11988.
27. Wilson AD, Stokes CR, Bourne FJ. Morphology and functional characteristics of isolated porcine intraepithelial lymphocytes. *Immunology* 1986, **59**, 109–113.
28. Butzner JD, Befus AD. Interactions among intraepithelial leucocytes and other epithelial cells in intestinal development and function. In: Lebenthal E, ed. *Human Gastrointestinal Development*. New-York, Raven, 1989, 749–775.
29. Schwartz R, Momburg F, Moldenhauer G, Dörken B, Schirrmacher V. Induction of HLA class-II antigen expression on human carcinoma cell lines by IFN-gamma. *Int J Cancer* 1985, **35**, 245–250.
30. Sollid LM, Kvale D, Brandtzaeg P, Markussen G, Thoorsby E. Interferon-gamma enhances expression of secretory component, the epithelial receptor for polymeric immunoglobulins. *J Immunol* 1987, **138**, 4303–4306.
31. Klein J, Figueroa F, Nagy ZA. Genetics of the major histocompatibility complex: the final act. *Annu Rev Immunol* 1983, **1**, 119–142.
32. Mostov KE, Friedlander M, Blobel G. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature* 1984, **308**, 37–43.
33. Paxton RJ, Mooser G, Pande H, Lee TD, Shively JE. Sequence analysis of carcinoembryonic antigen: identification of glycosylation sites and homology with the immunoglobulin supergene family. *Proc Natl Acad Sci USA* 1987, **84**, 920–924.
34. Hefta SA, Hefta LJF, Lee LTD, Paxton RJ, Shively JE. Carcinoembryonic antigen is anchored to membranes by covalent attachment to a glycosylphosphatidylinositol moiety: identification of the ethanolamine linkage site. *Proc Natl Acad Sci USA* 1988, **85**, 4648–4652.
35. Sack TL, Gum JR, Low MG, Kim YS. Release of carcinoembryonic antigen from human colon cancer cells by phosphatidylinositol specific phospholipase C. *J Clin Invest* 1988, **82**, 586–593.
36. Fantini J, Martin JM, Luis J, *et al.* Restricted localization of functional vasoactive intestinal peptide (VIP) receptors in *in vitro* differentiated human colonic adenocarcinoma cells (HT29-D4). *Eur J Cell Biol* 1988, **46**, 458–465.
37. Dharmsathaphorn K, Harms V, Yamashiro DJ, Hugues RJ, Binder HJ, Wright EM. Preferential binding of vasoactive intestinal peptide to basolateral membrane of rat and rabbit enterocytes. *J Clin Invest* 1983, **71**, 27–35.

**Acknowledgements**—We thank Fernand Giannellini and Jean-Jacques Roccabianca for expert technical assistance. This work was supported by the Fédération Nationale des Centres de Lutte Contre le Cancer (grant to J. M. and J. F.).

*Eur J Cancer, Vol. 27, No. 5, pp. 604–608, 1991.*
*Printed in Great Britain*

*0277–5379/91 $3.00 + 0.00*


# Colorectal Cancer in Northeast Italy: Reproductive, Menstrual and Female Hormone-related Factors

**Silvia Franceschi, Ettore Bidoli, Renato Talamini, Salvatore Barra and Carlo La Vecchia**

**The role of reproductive and menstrual factors and a few medical conditions linked to female hormones in the aetiology of colorectal cancer was investigated in a case-control study conducted in Pordenone province in northeastern Italy, on 89 women with colorectal cancer and 148 controls admitted to hospital for a wide spectrum of acute, non-digestive nor neoplastic disorders. After adjustment for age and social class, parous women, as compared to nulliparous ones, were significantly protected against colorectal cancer (odds ratio, OR = 0.4, [95% confidence interval, CI:0.2–0.8]) and the risk appeared to decrease with successive pregnancies up to five or more (0.2, [0.04–0.6]). Compared to women who had their first birth at age 24 or less, the OR for those who had it at 30 or older was 2.0, but the inverse trend in risk was not significant. However, among parous women only, age at first birth, but not parity, seemed to retain a certain influence. Late age at menopause seemed to decrease colorectal cancer risk (OR for menopause at age ≥50 vs. <45 = 0.4, [0.2–1.0] $\chi^2_1$ (trend) = 3.66). Conversely, age at last birth, number of abortions, years between marriage and first birth, age at menarche, pattern of menstrual cycle and occurrence of a few medical conditions potentially linked to female hormones were similarly reported by cases and controls. Due to the very limited number of oral contraceptive (OC) users (9 controls but only 1 case), and the lack of oestrogen replacement therapy users, the influence of exogenous female hormones on colorectal cancer could not be analysed meaningfully.**
*Eur J Cancer*, Vol. 27, No. 5, pp. 604–608, 1991

## INTRODUCTION

LONG-STANDING interest in the role of reproductive factors and female hormones in the aetiology of colorectal cancer stems from two major lines of evidence. Firstly, there are substantial similarities in the epidemiology of cancer of the colorectum and breast [1]. Incidence of and mortality from these neoplasms are positively correlated on both an international [2, 3] scale and within countries [4, 5]. Further, there are consistent and similar correlations between rates from these neoplasms and various indicators of modern affluence and/or intake of dietary fats and proteins [1, 6, 7]. Higher than expected incidence rates of colorectal cancer as well as breast, ovary and corpus uteri cancers have been noted in nuns [8], and in single women as compared to married women [9].