Human Cell Models To Study Small Intestinal Functions: Recapitulation of the Crypt-Villus Axis

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ABSTRACT The intestinal epithelium is continuously and rapidly renewed by a process involving cell generation, migration, and differentiation, from the stem cell population located at the bottom of the crypt to the extrusion of the terminally differentiated cells at the tip of the villus. Because of the lack of normal human intestinal cell models, most of our knowledge about the regulation of human intestinal cell functions has been derived from studies conducted on cell cultures generated from experimental animals and human colon cancers. However, important advances have been achieved over recent years in the generation of normal human intestinal cell models. These models include (a) intestinal cell lines with typical crypt cell proliferative noncommitted characteristics, (b) conditionally immortalized intestinal cell lines that can be induced to differentiate, and (c) primary cultures of differentiated villuslike cells that can be maintained in culture for up to 10 days. Each of these models should help in the investigation of the specific aspects of human intestinal function and regulation. Furthermore, taken together, these models provide an integrated system that allows an in vitro recapitulation of the entire crypt-villus axis of the normal human small intestine. Microsc. Res. Tech. 49:394–406, 2000.

INTRODUCTION

The intestinal epithelium is continuously and rapidly renewed by a process involving cell generation and migration from the stem cell population located at the bottom of the crypt to the extrusion of the terminally differentiated cells at the tip of the villus, 3–5 days later (Babaysky and Podolsky, 1999). This highly dynamic system appears particularly well suited for studying key biological phenomena such as cell proliferation, migration, and tissue-specific gene expression. Its functional unit, the crypt-villus axis, which develops relatively early during human ontogeny (being established by midpregnancy), can be defined by typical morphological and functional properties displayed by the mature villus enterocytes that distinguish them from the crypt cells. Indeed, villi are mainly lined by functional absorptive and goblet cells whereas the crypts contain stem cells, poorly differentiated and proliferative cells, and a subset of differentiated secretory cells, the Paneth cells. The compartmentalization of distinct cell populations according to their functional state can be exemplified by analyzing the distribution of various markers along the crypt-villus axis (Fig. 1). In all species studies, the crypt-villus junction represents a physical limit from which enterocytes acquire their final functional characteristics. For instance, lactase-phlorizin hydrolase and maltase-glucosamylase complexes, which are markers of functional enterocytes in the developing and adult small intestine, respectively, are restricted to the villus; the proliferating antigen Ki67 and the specific secretory granule marker MIM-1/39 are expressed only by crypt cells (Louvard et al., 1992; Ménard and Beaulieu, 1994). However, it becomes more and more evident that in the human, in contrast with the situation observed in laboratory animals, some of the classical enterocyte markers can be expressed by immature cells located below this border. For instance, aminopeptidase N and dipeptidylpeptidase IV have been found to be constitutively expressed in both proliferative and differentiated human intestinal cells (Ménard and Beaulieu, 1994). The immature forms of sucrase-isomaltase and apolipoprotein B are present in adult and fetal intestinal crypt cells, respectively (Basque et al., 1998; Beaulieu et al., 1989).

Gene expression in intestinal cells must be tightly regulated to orderly control cell proliferation, migration, and differentiation along the crypt-villus axis. The mechanisms are still incompletely understood but, likely, include several regulatory factors such as hormones, growth factors, and cytokines as well as cell-cell and cell-matrix interactions (Beaulieu, 1999; Gordon and Herminston, 1994; Montgomery et al., 1999; Pignatelli et al., 1997; Podolsky, 1993). Because of the unavailability of normal human intestinal epithelial cell models, most of our knowledge about human intestinal cell regulation has been derived from studies conducted on cell cultures generated from experimental animals (Evans et al., 1994; Kedinger et al., 1987; Quaroni and May, 1980) and human colon cancers (Ménard and Beaulieu, 1994; Moyer et al., 1990; Rousset, 1986; Whitehead and Watson, 1997; Zweibaum and Chantret, 1989). The
utility, as well as the limitations, of these models is well documented. On one hand, rodent intestinal cell models have proven to be useful for analyzing intestinal cell properties, including the regulation of proliferation, migration, synthesis of extracellular matrix molecules, and tissue-specific gene expression (Boudreau et al., 1996; Carroll et al., 1988; Conteas and Majumdar, 1987; Evans et al., 1994; Kurokawa et al., 1987; Paul et al., 1993; Quaroni et al., 1978; Ruemmele et al., 1999a). However, an important constraint with these systems is that observations performed with experimental animal models cannot always be transposed to the human. This interspecies variability has been illustrated above in the case of brush border enzyme expression. More importantly, it is also apparent for the regulation of gene expression, both during development and along the crypt-villus axis, which seems to differ fundamentally between human and animal models (Babjatsky and Podolsky, 1999; Beaulieu, 1999; Boyle and Brenner, 1995; Chailler and Ménard, 1999; Ménard and Beaulieu, 1994). On the other hand, human colon cancer cell lines have been used to investigate various aspects of intestinal cell function and regulation. Although some of them have been used advantageously for a number a purposes (see below), the theoretical limitations with these models are obviously their cancerous nature and their colonic origin (Ménard and Beaulieu, 1994; Moyer et al., 1990; Rousset, 1986; Whitehead and Watson, 1997; Zweibaum and Chantret, 1989).

Recent progress in the generation of normal cell models has provided new tools to study human intestinal cell functions without these limitations. Among them, the HIECs (Perreault and Beaulieu, 1996), a series of human intestinal cell lines with typical crypt cell proliferative characteristics; the tsFHIs (Quaroni and Beaulieu, 1997), a set of conditionally immortalized fetal human intestinal cell lines with a temperature-sensitive SV40 large T antigen, which can be induced to undertake differentiation; and the PCDEs (Perreault and Beaulieu, 1998), which are fully differentiated enterocytes that can be maintained in primary culture for about 10–12 days. In this review, after a brief overview of the human intestinal cell systems currently used with a specific emphasis on the Caco-2 cell line, we will summarize the characteristics of these new models and provide the evidence that taken together, these models allow for an in vitro recapitulation of the entire crypt-villus axis of the normal human intestine.

**Fig. 1.** The crypt-villus functional unit of the human small intestine. In this rapidly renewing epithelium, the proliferative cells, which arise from the stem cells located at the bottom of the crypts, lose their ability to proliferate. They start to differentiate in the upper third of the crypt, then migrate toward the tip of the villus, before being exfoliated into the lumen. Functional markers of the distinct cell populations include the proliferating Ki67 antigen, the cyclin-dependent kinase inhibitor p21WAF/CIP1 (p21), the crypt cell antigen MIM-139 (MIM39), dipeptidases (DP), and disaccharidases (DS). (Adapted from Beaulieu, 1997, 1999, with permission.)
COLON ADENOCARCINOMA CELL LINES

For more than two decades, human colorectal cancer cell lines have been proven to be useful experimental model systems to help better understand the underlying biological and molecular basis of colon cancer (Hague et al., 1997; Rutzky and Moyer, 1990). Interestingly, among the more than 100 adenocarcinoma cell lines that have been derived from the human colon and further characterized under various culture conditions, only a few of them (e.g., HT29, HRA-19, T84, and LIM1963) have been found to exhibit some biochemical and/or morphological properties associated with the small intestinal epithelium (Kirkland, 1986; Madara et al., 1987; Pinto et al., 1982; Whitehead et al., 1987). Only one, Caco-2, spontaneously exhibited typical enterocytelike differentiation characteristics (Pinto et al., 1983). Indeed, under standard culture conditions, Caco-2 cells form a confluent monolayer. Over a period of 20–40 days of postconfluent culture, Caco-2 cells gradually acquire full morphological polarity with junctional complexes and a typical brush border (Fig. 2), as well as relatively high levels of brush border hydrolases including sucrase-isomaltase (Beaulieu and Quaroni, 1991; Hauri et al., 1985; Pinto et al., 1983; Vachon and Beaulieu, 1992). The issue of expression of small intestinal characteristics such as sucrase-isomaltase and lactase in cell lines established from the colon has been addressed in detail. The conclusion is that these cells exhibit similar features to those found in the human fetal intestinal epithelium, which before 20–22 weeks, is similar in both small intestine and colon (Ménard and Beaulieu, 1994; Roussel, 1986; Zweibaum and Chantret, 1989).

The potential value of Caco-2 cells in the study of the various aspects of human small intestinal cell physiology is well documented (Beaulieu et al., 1989; Blais et al., 1987; Hauri et al., 1985; Levy et al., 1995; Roussel et al., 1989). The cell line has also been shown to represent an excellent model to study cell differentiation and its regulatory mechanisms. This is well exemplified by studies on intestinal brush border hydrolase expression, namely, sucrase-isomaltase. These studies have provided evidence for both transcriptional and posttranslational control sites (Beaulieu et al., 1989; Chantret et al., 1994; Quaroni et al., 1993; Sebastio et al., 1987), dependence on MAPKinease activities (Aliaga et al., 1999), modulatory influences by growth factors (Beaulieu and Quaroni, 1991; Cross and Quaroni, 1991; Zhou et al., 1998), and cell-matrix interactions (De Archangelis et al., 1996; Vachon and Beaulieu, 1995; Vachon et al., 1995; Fig. 3). However, their analysis at the cellular level revealed an intriguing property of these cells: a remarkable and transitory heterogeneity of their differentiation characteristics. Indeed, these studies showed that in Caco-2 cells, the expression of most brush border enzymes, morphology, and brush border organization greatly vary from cell to cell in the monolayer during the first 20 days of postconfluent culture (Vachon and Beaulieu, 1992; Vachon et al., 1996). Furthermore, this mosaic pattern of differentiation was maintained in clones and subclones of the Caco-2 cell line (Beaulieu and Quaroni, 1991; Vachon and Beaulieu, 1992) and was found to proceed uncoupled relative to the expression of the various markers studied (Vachon et al., 1996). This still incompletely understood mosaic and uncoupled pattern of Caco-2 cell differentiation has to be considered when using this model.

INTESTINAL CELL MODELS DERIVED FROM THE NORMAL HUMAN SMALL INTESTINE

The ideal model for investigating the mechanisms that regulate intestinal cell growth and differentiation would be an in vitro culture model of permanent normal cells that can be induced to fully differentiate under strictly controlled conditions, a sort of "normal Caco-2 counterpart." Although this goal has been achieved to some extent for the hemopoietic system (Lemischka, 1999; Srou et al., 1999) and the epidermis (Terskikh and Vasiliev, 1999), attempts to generate such systems for the human intestinal epithelium have met with very limited success. The major problems are poor survival of the epithelial cell population and stromal cell overgrowth. Various strategies were undertaken to overcome these difficulties. One of them was to use fetal tissue for these experiments because it can be obtained under sterile conditions and because younger tissues appear more effective in the generation of epithelial cell cultures (Evans et al., 1994). Furthermore, as mentioned above, in humans, midgestation fetal cells express most of the differentiated characteristics typical of the adult small intestine.

HIEC: Undifferentiated Stemlike Cells

A number of isolation procedures, principally developed for the rodent intestine (Evans et al., 1994; Moyer et al., 1990; Quaroni and May, 1980), were tested in order to obtain viable human enterocytes free of mesenchymal cells. Mechanical dissociation methods were found to allow only limited epithelial cell recovery and short-term viability. All assays based on enzymatic dissociation using collagenase, dispase, and pronase, alone or in combination, generated cultures rapidly overgrown by fibroblasts (Perreault and Beaulieu, 1996). However, thermolysin, a dissociating enzyme successfully used for keratinocyte isolation (Germain et al., 1993), considerably improved the yield of growing mesenchymal-free intestinal epithelial cells (Perreault and Beaulieu, 1996). Interestingly, the treatment was particularly efficient when using 17- to 19-week-old ileum, cell recoveries being much lower with younger tissues, whereas myofibroblast contamination was more frequent when using jejunum or older ileum specimens.

The morphological and functional characterization of these HIEC provided evidence that they are comparable to the undifferentiated cells of the crypts (Perreault and Beaulieu, 1996). Like their rodent IEC-6 counterpart (Quaroni and May, 1989), the HIEC exhibit an undifferentiated epithelial morphology (Fig. 4A). Their epithelial origin was confirmed by identification of the intestinal keratins 8, 18, 19, and 20/21 in Western blotting (Fig. 4B) and immunostaining as well as components specific for desmosomes and tight junctions. Analysis of the expression of intestinal functional markers provided further evidence of the undifferentiated nature of the HIEC. Indeed, these cells retain the ability to express the 350-kD crypt cell-specific marker
MIM-1/39 (Beaulieu et al., 1992; Calvert et al., 1993) as well as the brush border hydrolases aminopeptidase N and dipeptidylpeptidase IV, but not sucrase-isomaltase (Perreault and Beaulieu, 1996). The lack of sucrase-isomaltase at both the protein and transcript levels (Quaroni and Beaulieu, 1997; Fig. 5), in concert with poorly morphological enterocytic differentiation features, is indicative of the noncommitted nature of these cryptlike cells. This possibility was further emphasized by the fact that growing HIEC on a laminin-1 substrate failed to significantly enhance their polarization characteristics and expression of intestinal cell markers (Fig. 4C,D) although their adhesion and spreading appear to be integrin dependent (Fig. 4E).

Fig. 2. Morphological differentiation of Caco-2/15 cells in standard culture conditions. The analyses of the monolayer by light microscopy (A–C,E) and transmission (D,F) and scanning (G,H) electron microscopy reveal that subconfluent (A) and newly confluent (B) Caco-2 cells are poorly polarized. However, soon after confluence, the monolayer appears better organized with a more cuboidal cell shape (C) and cell junctions and microvilli (D). When complete structural polarization has been achieved, after 30 days, the cells display a typical cylindrical enterocytelike morphology (E) with a well-developed brush border (F–H). A–C,E: 668×; D: 3,636×; F: 4,121×; G: 1,273×; H: 25,212×. (Adapted from Vachon and Beaulieu, 1992, with permission.)
The generation of continuously growing HIEC cultures that retain the ability to behave like intestinal crypt cells for a significant number of passages provides an important new tool to investigate, in vitro, this still poorly understood cell population of the human small intestine. Recent studies from our laboratory and elsewhere have shown that HIEC are useful in analyzing the regulation of cell proliferation (Perreault and Beaulieu, 1996; Ruemmele et al., 1998). They have also been proven to be a useful model to study the mechanism of FAS-induced apoptosis (Ruemmele et al., 1999b), the functional basis of cell matrix interactions (Basora et al., 1999; Desloges et al., 1998; Simoneau et al., 1998) as well as lipid metabolism (Levy et al., 2000).

By analogy, rodent IEC-type cells also remain, morphologically and functionally, poorly differentiated in culture (Quaroni and May, 1980). Evidence of stem cell properties has been elegantly demonstrated by in vivo grafting experiments (Kedinger et al., 1986) and forced expression of the cdx-2 gene (Suh and Traber, 1996). They have also been proven to be a useful model to study the mechanism of FAS-induced apoptosis (Ruemmele et al., 1999b), the functional basis of cell matrix interactions (Basora et al., 1999; Desloges et al., 1998; Simoneau et al., 1998) as well as lipid metabolism (Levy et al., 2000).

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**tsFHI Cell Lines: Differentiating Cryptlike Cells**

Intestinal cell models recapitulating the well-coordinated processes that control proliferation and differentiation as they occur in the intact upper crypts (Fig. 1) are difficult to establish in vitro. As summarized above, conventional methods for intestinal epithelial cell isolation have only allowed the isolation of either proliferative but noncommitted cells such as the HIEC or very short lived and quiescent differentiated cells. This suggests that under these in vitro conditions, committed intestinal cells spontaneously undertake their differentiation program and irreversibly lose their proliferative activity (Gordon and Hermiston, 1994). In such a case, one potentially powerful strategy may be the immortalization of primary cultures with transforming oncogenes, such as the SV40 large T-Ag, adenovirus E1A/E1B, and papillomavirus E6/E7. They have the ability to induce DNA synthesis and prolonged growth of host cells (Nevins, 1994). However, the common drawback that has been observed using wildtype oncogenes is the substantial reduction in the expression of differentiation features in immortalized intestinal cells (Chastre et al., 1993; Emami et al., 1989; Whitehead et al., 1993). This problem was partially overcome by using a temperature-sensitive mutant of the SV40 T-Ag to derive conditionally immortalized intestinal cell lines from fetal rat at early stages of development (Paul et al., 1993). These cells grow at 32°C but quickly lose proliferative activity and revert to a normal phenotype when switched to the nonpermissive temperature (39°C). The method, however, was not applicable to the more mature intestinal cells present after 18 days of gestation. Levels of differentiation reached appeared variable, depending on the culture parameters (Paul et al., 1993). The method developed by Paul et al. (1993) was adapted for the fetal human small intestine (Quaroni and Beaulieu, 1997). It was reasoned that, being much more structurally and functionally mature than the fetal rat intestine, the human small intestinal epithelium at 16–19 weeks of gestation would be more likely to produce cell lines that retain a higher level of differentiation. Another key element of the method was the optimization of both the growth and differentiation media to maximize transformation efficiency, cell
growth, and differentiation (Quaroni and Beaulieu, 1997).

The intestinal origin and the morphological and functional characteristics of the tsFHI cells have been analyzed in great detail (Quaroni and Beaulieu, 1997). At the permissive temperature, mitoses were relatively frequent and the population doubling time was estimated at 5–7 days. All clones were found to express the

![Morphological and functional characterization of HIEC cells. A: HIEC cells have an undifferentiated epithelial morphology, as displayed in phase contrast and electron microscopy. B: Western blotting experiments confirmed the intestinal epithelial origin of HIEC as illustrated by the presence of keratin 8, 18, and 20/21 (K8, K18, k21) and the expression of the MIM-1/39 crypt cell antigen (lane 1). Compare with intestinal mesenchymal cells (lane 2), fetal human small intestine (lane 3), and Caco-2 cells (lane 4). C–E: Plating the HIEC cells on laminin-1 has little effects on the ultrastructural (C) and functional (D) differentiation, although it involves an integrin-mediated adhesion process as illustrated by a substantial and specific activation of the focal adhesion kinase (FAK) after 30 minutes on laminin-1 (E). (A,B: Adapted from Perreault and Beaulieu, 1996, with permission; C,D: unpublished data from Perreault et al.; E: unpublished data from Basora et al.).]
same intestinal cell markers as those identified in HIEC, including the main intestinal keratins, the brush border enzymes aminopeptidase N and dipeptidylpeptidase IV, and the crypt cell marker MIM-1/39. They displayed a generally flat morphology (Fig. 5A). Switching the tsFHI cells to the nonpermissive temperature produced marked morphological and functional changes consistent with the acquisition of a more differentiated phenotype. This included a rapid, complete, and irreversible growth arrest associated with a strong induction of the cyclin-dependent kinase inhibitor p21WAF/Cip1. There was also a gradual acquisition of a more regular and polygonal shape associated with the redistribution of junctional complex proteins at the cell-to-cell contacts, the appearance of a dense layer of microvilli (Fig. 5B), significant increases in the expression of aminopeptidase N and dipeptidylpeptidase IV (Fig. 5C) and induction of sucrase-isomaltase expression (Fig. 5D).

From these observations, it was concluded that tsFHI cells represent a new and representative model for the study of intestinal cell differentiation in vitro, one that would be comparable to the processes taking place in the intestinal crypts in vivo (Quaroni and Beaulieu, 1997).

Another interesting characteristic of the tsFHI cell model is that two phases in the differentiation process could be experimentally defined and, more important, investigated as separated phenomena: an early phase characterized by the irreversible loss of proliferation and a late phase in which morphological and functional differentiation features take place (Quaroni and Beaulieu, 1997). The usefulness of the model was recently demonstrated (Tian and Quaroni, 1999). In their study, the expression of cell cycle regulatory proteins in tsFHI cells during growth and the early and late phases of differentiation implicated the cyclin-dependent kinase inhibitors p21WAF/Cip1 and p27Kip in the intestinal dif-

Fig. 5. Morphological and functional characterization of tsFHI cells. A,B: Scanning electron microscopy shows that tsFHI cells grown at the permissive temperature have a flat morphology (A). Cells maintained for 6 days at the nonpermissive temperature display a more tight, cobblestone appearance and are covered with a much denser layer of apical microvilli (B). C,D: These morphological changes are accompanied by a substantial increase in the expression of the brush border enzyme dipeptidylpeptidase IV, starting 3 days after the shift to the nonpermissive temperature (C) and the induction of sucrase-isomaltase expression, as determined by reverse transcription-polymerase chain reaction (D, lanes 3,4). A,B: 2,100×. (A,B,D: Adapted from Quaroni and Beaulieu, 1997, with permission; C, unpublished data from Pageot et al.)
differentiation process, with temporally and functionally distinct roles.

**PCDE Cultures: Fully Differentiated Villuslike Cells**

In vitro models of fully differentiated normal enterocytes like those found on the villus are still lacking. Indeed, because of their nonproliferative status, normal differentiated enterocytes can only be maintained in vitro as primary cultures. As mentioned above, the usefulness of these cultures has been limited by low recoveries, fibroblast contamination, very limited survival time, and poor maintenance of the differentiated phenotype (Fonti et al., 1994; Perreault and Beaulieu, 1996) unless a mesenchymal support is provided (Sanderson et al., 1996). A possible explanation for this phenomenon is that conventional dissociation methods used to separate epithelial cells from their extracellular matrix support may rapidly induce permanent cellular damage (Grossman et al., 1998; Sträter et al., 1996). However, we have recently described a new approach to isolate viable villus epithelial cells from the fetal human small intestine (Perreault and Beaulieu, 1998). The procedure was based on the use of Ma-

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**Fig. 6. Generation of primary cultures of differentiated enterocytes.** A–C: Phase-contrast micrographs of the luminal aspect of the human fetal small intestine following a 9-hour incubation period in Matrisperse solution. Before agitation, the epithelium remains in place (A). After gentle agitation, the entire epithelial lining becomes completely dissociated (C) from the remaining underlying mesenchyme (B). D: Western blot analysis of the intact (I) intestine and isolated mesenchymal (M) and epithelial (E) fractions for the presence of sucrase-isomaltase (SI), E-cadherin (E-cad), keratin 18 (K18), vimentin (Vim), and α-smooth muscle actin (αSMA) confirmed the relative purity of both epithelial and mesenchymal fractions. E,F: Phase-contrast microscopy showing the epithelial fraction in culture. After 24 hours, the villuslike epithelium fragments have adhered and initiated the spreading (E), which will continue until confluence is reached after an additional 2–3 days (F). Confluent primary cultures of differentiated enterocytes can be maintained for about 10–12 days before observing the first signs of degeneration (G). A–C: 12×; E–G: 45×. (Adapted from Perreault and Beaulieu, 1998, with permission.)
trisperse (Collaborative Biomedical Products, Bedford, MA; Becton Dickenson Labware, Mississauga, Ontario, Canada), a nonenzymatic solution initially designed to recover epithelial cells grown on Engelbreth-Holm-Swann (EHS) biomatrix. Incubation of small intestinal fragments in Matrisperse at 4°C without agitation for 8–12 hours produces little apparent effect on the villus structure, other than a swollen appearance (Fig. 6A). Surprisingly, gentle agitation of the medium results in a complete dissociation of the entire epithelial lining (Fig. 6C) from the underlying mesenchyme (Fig. 6B). Analyses of the isolated epithelial and remaining mesenchymal fractions for the expression of tissue-specific markers such as sucrase-isomaltase, E-cadherin, keratin 18, vimentin, and α-smooth muscle actin illustrate the relative purity of both epithelial and mesenchymal fractions (Fig. 6D). Further analyses at the transcript level confirmed these observations and provided evidence on the usefulness of the isolation method to determine the epithelial and mesenchymal contributions to the intestinal basement membrane composition (Perreault et al., 1998).

Interestingly, plating the epithelial fractions on culture dishes, coated with rat-tail collagen to improve adhesion, showed a very good viability of these preparations. As illustrated in Figure 6, villuslike remnants...
adhere and epithelial cells begin rapidly to spread on the substrate (Fig. 6E) and continue to do so until confluence is reached, 3–4 days later (Fig. 6F). At 5–6 days after plating, the cultures still appear as typical monolayers and can be maintained as such for an additional 5 days before observing the first sign of degeneration (Fig. 6G). Morphological and functional characteristics of PCDE 6–7 days after the plating are summarized in Figure 7. Electron microscopy and indirect immunofluorescence analyses revealed that the PCDE are composed of both absorptive and goblet cells exhibiting ultrastructural characteristics and functional properties similar to those found in the intact villus epithelium (Fig. 7A–H). Furthermore, DNA synthesis in PCDE was found to be negligible 48 hours after plating (Fig. 7I), at a stage when colonies are rapidly expanding. PCDE thus appear to be a unique in vitro system of villuslike intestinal cell culture (Perreault and Beaulieu, 1998).

Based on their morphological and functional properties, PCDE represent a normal counterpart for the widely used colon adenocarcinoma cell models such as Caco-2 and HT-29 cells in their differentiated state (see above). Because PCDE are relatively easy to reproduce, there are numerous potential uses. In addition to the study of normal enterocytic absorptive and digestive functions in vitro, PCDE may allow the determination of the effects of growth factors and cytokines and the role of cell-cell and cell-matrix interactions on a normal differentiated nonproliferating intestinal cell population. For instance, because of their nonproliferating status, PCDE provide an interesting tool for studying intestinal restitution, a healing process currently studied with intestinal cell lines (Ciacci et al., 1993; Lotz et al., 1997), but that does not require cell proliferation (Dignass and Podolsky, 1993; Poulsom, 1997). Other applications for PCDE may include the analysis of drug and nutrient transport and metabolism as a normal alternative to the widely used and apparently controversial (Barthe et al., 1999; Delie and Rubas, 1997) Caco-2 cell line.

CONCLUSIONS AND PERSPECTIVES

The regulation of intestinal functions has been the subject of numerous studies. In humans, progress in this field has been traditionally hampered by the lack of normal epithelial cell models. However, we know more about the intestinal regulatory factors such as growth factors and cytokines (Chaillet and Menard, 1999; Podolsky, 1993) and epithelial-mesenchymal and cell-matrix interactions (Beaulieu, 1997, 1999; Kedinger et al., 1999). We have a better understanding of how to use oncogenes to manipulate DNA synthesis and prolong the growth of host cells (Nevins, 1994), combined with the development of new epithelial cell isolation methods (Germain et al., 1993; Moyer et al., 1990; Whitehead et al., 1993) and improvements in culture media formulation. All these factors likely contributed to the recent advancement in the development of the new human intestinal cell models presented in this study.

As summarized in Table 1, each of these models appears to be representative of one of the main functional compartments in the intact intestinal crypt-villus axis. First, intestinal cell lines with typical proliferative cell characteristics such as the HIEC, like their rodent counterpart (Quaroni and May, 1980), are unable to differentiate. They are poorly polarized and express only low levels of dipeptidases. Taken in conjunction with their apparent non-committed status, it suggests that HIEC behave as stem-like cells representative of the bottom of the crypt. Second, the conditionally immortalized tsFHI intestinal cells are phenotypically comparable to HIEC when grown under permissive conditions. However, under nonpermissive conditions, they acquire morphological and functional characteristics similar to those found in the upper half of the crypt including loss of proliferative capacity and sucrase-isomaltase expression. Third, the villus-like PCDE exhibit the same characteristics as those found in the intact villus epithelium and can be maintained in vitro for more than 10 days. Taken together, these three models allow a fairly accurate in vitro reproduction of the entire human normal crypt-villus axis (Fig. 8).

The combination of these three models has the benefit of providing, for the first time, a new tool for investigating the regulatory mechanisms of cell growth and differentiation in the normal human intestinal epithelium. One may argue that it may not yet correspond exactly to the perfect system, principally because of its relative complexity. However, far from being a weakness, this particularity appears quite advantageous. It allows the experimental separation of the different cell states that occur along the crypt-villus axis. Indeed, in currently used experimental models, such as Caco-2 cells, the loss of proliferation and acquisition of differ-

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TABLE 1. Summary of the properties of the various intestinal cell models as compared to the fetal intestinal epithelium

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<th>Model</th>
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<th>Caco-2 PC</th>
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1PC, postconfluent; FIE, fetal intestinal epithelium; n.d., not determined.
2Irreversibly nonproliferative.
3Reversible nonproliferative status.
entiation characteristics appears as a gradual and heterogeneous phenomenon (see above). In contrast, proliferation, growth arrest, initiation of differentiation, and the fully differentiated state can now be studied separately.

The potential applications for each of these models are numerous and have been specifically addressed in this study. Some of them are directed to the characterization of particular features associated with a specific cell stage. For example, undifferentiated crypt cells have the ability to synthesize and secrete lipid and apolipoproteins (Levy et al., 2000) or to express specific forms of integrins (Basora et al., 1997, 1999; Desloges et al., 1998). Another field that should benefit from the development of these models is the identification and characterization of the regulatory mechanisms of intestinal cell growth, differentiation, and survival. Finally, the field of intestinal nutrient and drug transport and metabolism should also benefit from these models, namely the PCDE. Although the use of PCDE may be limiting on a large scale for this purpose, it could be advantageously used as a validating system for the currently used colon adenocarcinoma models. Indeed, it has to be kept in mind that under many instances, Caco-2 and some other cell lines, albeit their cancerous origin, appear to behave similarly to their normal in vivo counterpart, particularly under the differentiated state (see Table 1 for Caco-2 cells). Thus, one additional outcome of the development of these new models, particularly the PCDE, may be to eventually reinforce the use of cancer cell lines as experimental models, by permitting the validation of them being phenotypically normal.

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