In Vitro Cell. Dev. Biol.—Animal 34:660–664, September 1998

1071-2690/98 $05.00+0.00

FACTORS AFFECTING CACO-2 INTESTINAL EPITHELIAL CELL INTERLEUKIN-6 SECRETION

S. J. D. VITKUS, S. A. HANIFIN, AND D. W. McGEE[1]

Department of Biological Sciences, Binghamton University (SUNY), Binghamton, New York 13902-6000

(Received 11 December 1997; accepted 22 April 1998)

SUMMARY

Intestinal epithelial cells (IEC) have previously been shown to produce several cytokines including interleukin-6 (IL-6). However, many factors which may regulate IL-6 secretion by human IEC still remain a mystery due in part to the lack of appropriate model cell lines and the difficulty of culturing human IEC over long periods of time. We have determined that the human colonic carcinoma cell line Caco-2 is capable of secreting IL-6 when stimulated by the inflammatory cytokines IL-1β or tumor necrosis factor-α (TNF-α), and stimulation of these cells with IL-1β plus TNF-α induced a synergistic enhancement of IL-6 secretion. The inflammatory cytokine-induced enhancement in IL-6 secretion was greatest when the cells were cultured in a 10% CO_2 atmosphere as compared to cells grown in 5% CO_2, suggesting that environmental CO_2 levels may affect IEC cytokine secretion. Finally, long-term culture of the Caco-2 cells to induce cellular differentiation had no effect on the capacity of these cells to produce IL-6, indicating that the regulation of IL-6 secretion was not affected by differentiation. Taken together, these studies provide important information on the factors which regulate IL-6 secretion by human IEC as they may contribute to the cytokine network during a mucosal inflammation. The results also suggest that the Caco-2 cell line is an appropriate model for further studies on the regulation of cytokine secretion by human IEC.

Key words: cytokine; colonic; CO_2; differentiation; human.

INTRODUCTION

The intestinal epithelial cells (IEC) line the intestine and colon and form a barrier between the contents of the gut lumen and the underlying tissues. These cells are therefore in a position to provide initial alarm signals, via secreted cytokines, for microbial invasion or the presence of toxins in the gut lumen. Intestinal epithelial cells have been shown to be capable of producing a variety of cytokines including interleukin-1 (IL-1), IL-6, IL-8, transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α) (Eckmann et al., 1993; Jung et al., 1995; McGee and McGhee, 1996; Weinstein et al., 1997). Of these cytokines, IL-6 plays an important role in the induction of the acute phase response and enhancing the inflammatory response (Akira et al., 1993). IL-6 is also capable of enhancing B cell immunoglobulin secretion, particularly IgA, and acts as a costimulator for T cell proliferation (Akira et al., 1993) which could enhance the local intestinal mucosal immune response. *In vivo*, small intestinal and colonic epithelial cells have been shown to express IL-6 (Shirota et al., 1990; Jones et al., 1993). More importantly, several reports have shown colonic epithelial cells from patients with inflammatory bowel diseases produce IL-6 protein or mRNA (Jones et al., 1993; Woywodt et al., 1994; Kusugami et al., 1995) suggesting an importance for this IEC-derived cytokine in mucosal pathogenesis.

Although IEC appear fully capable of producing IL-6, the mechanisms that regulate IL-6 expression by these cells are still incompletely understood. Normal IEC are difficult to prepare without contamination from intraepithelial lymphocytes or lamina propria cells, and isolated normal IEC rarely survive beyond 24 h in culture. Still, using short-term culture, investigators have found that normal human IEC can be induced to secrete IL-6 by bacterial invasion (Jung et al., 1995) and by stimulation with the inflammatory cytokine IL-1β (Jung et al., 1995; Panja et al., 1995). Some further studies on the regulation of IL-6 secretion by IEC have been accomplished with cultured intestinal epithelial cell lines. We have used the nontransformed rat small intestinal epithelial cell line IEC-6 in several studies which suggest that IEC can be induced by IL-1β, TNF-α, or TGF-β and combinations of these cytokines to secrete high levels of IL-6 (McGee et al., 1992, 1993, 1995). Although nontransformed human crypt-like IEC cell lines have recently been developed which also appear capable of producing IL-6 and show much promise for future studies (Pang et al., 1996), no studies to date with human IEC have been able to confirm other aspects of our studies with the rat IEC-6 cells. In addition, it is unclear whether small intestinal and colonic IEC differ in their regulation of IL-6 secretion. Indeed, several human colonic IEC lines have been shown to be unable to produce IL-6, even when stimulated with cytokines or bacteria (Eckmann et al., 1993; Jung et al., 1995). And finally, the IEC-6 cell line used in our studies and the recently developed human IEC lines are both immature crypt-like IEC (Quaroni et al., 1979; Pang et al., 1996) and, therefore, virtually nothing is known about the effect of IEC differentiation on the regulation of IL-6 secretion.

In this study, we have examined the capacity of the human colonic carcinoma cell line Caco-2 to produce IL-6 with cytokine stimulation. Previously, other reports have suggested that Caco-2 cells do not

[1]To whom correspondence should be addressed.

produce IL-6 when stimulated by inflammatory cytokines, e.g., IL-1β and TNF-α or bacteria such as *Escherichia coli* (Hedges et al., 1992; Eckmann et al., 1993; Jung et al., 1995). In contrast, a more recent study by Weinstein and colleagues (1997) showed that Caco-2 cells can secrete significant levels of IL-6 when infected by the enteric pathogen *Salmonella typhi*. The present study confirms and extends these latter findings by demonstrating that the inflammatory cytokines IL-1β and TNF-α can induce Caco-2 cells to secrete significantly elevated levels of IL-6 under appropriate conditions. Further, the cytokine requirement for the induction of IL-6 secretion by these cells was found to be similar to that of the rat small intestinal IEC-6 cells, indicating that human IEC may be regulated in a similar fashion. Finally, utilizing the unique characteristic of the Caco-2 cells to undergo spontaneous differentiation to a villus-like IEC (Pinto et al., 1983), we were able to study the effect of differentiation on IL-6 secretion. These studies suggest that the Caco-2 cell line may be an excellent model to further study the regulation of cytokine secretion by human IEC and provide the basis for future detailed studies of this regulation.

Materials and Methods

Cell culture. The human colonic adenocarcinoma cell line Caco-2 (HTB 37) was purchased from the American Type Culture Collection (Rockville, MD). The cells were cultured in RPMI-1640 (Mediatech, Washington, D.C.) supplemented with 10% fetal bovine serum (FCS; HyClone Laboratories, Logan, UT), 10 m*M* L-glutamine, 10 m*M* nonessential amino acids, and 25 U penicillin per ml and 25 μg streptomycin (Mediatech) per ml designated as 10% RPMI Complete medium. All cells were grown at 37° C in a 90% air-10% CO_2 humid atmosphere unless otherwise noted. The cells were determined to be mycoplasm-free by staining with the Hoechst 33258 staining technique (Chen, 1977).

The basic culture method of the Caco-2 cells for IL-6 production was as follows. Cultures were initiated at 2×10^5 cells/well in 12-well tissue culture plates with 10% RPMI Complete medium. After 2 d, the adherent cells were washed with serum-free medium and then 1.5 ml of fresh 1% fetal calf serum (FCS)-containing medium with the appropriate cytokines was added. The cultures were then incubated for an additional 48 h with the stimulation. The culture supernatants were then collected and stored frozen at −80° C for determination of secreted IL-6 levels. The remaining adherent cells were removed by trypsin and EDTA treatment (Sigma Chemical Co., St. Louis, MO) and counted with a hemacytometer.

In some experiments, the effect of CO_2 atmosphere concentration on IL-6 secretion was tested. The Caco-2 cells from two similar liquid nitrogen frozen samples were cultured in 75-cm² flasks with 10% RPMI Complete medium supplemented with 50 m*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and placed in incubators with either a 5% or 10% CO_2 atmosphere. After culturing the cells to confluence, the cells were harvested and added at 4×10^5 cells/well in 24-well culture plates in 10% RPMI Complete medium with HEPES. After 7 d, the medium was replaced with fresh medium containing the appropriate cytokines and the cultures were incubated 48 h for cytokine secretion. The supernatants were then collected for IL-6 determination and the cells counted as above.

Determination of secreted IL-6 levels. Culture supernatants were assayed for IL-6 content by a bioassay with the IL-6-dependent 7TD1 mouse hybridoma (Van Snick et al., 1986; McGee et al., 1992). Aliquots of 2×10^3 7DT1 cells in RPMI 1640 containing 10% FCS, L-glutamine, antibiotics, 10 m*M* HEPES and 5×10^{-2} *M* 2-mercaptoethanol were added to serial twofold dilutions of the culture supernatants in 96-well tissue culture plates and incubated at 37° C. A standard of recombinant human (rh) IL-6 (R&D Systems, Minneapolis, MN) was also prepared in a similar manner. After 4 d, the proliferation of the cells was determined with an MTT colorimetric assay (Mosmann, 1983). The resulting purple crystals from the MTT assay were then solubilized with 200 μl of acidified isopropanol and mixed, and the absorbance of the samples was measured at 570 nm–650 nm with a BioTek EL312e microplate reader (Winooski, VT). The concentration of IL-6 in the samples was determined by a comparison to the rhIL-6 standard curve which was linear between 0.2 and 39 pg/ml.

Isolation of brush border membranes and determination of alkaline phosphatase levels. Caco-2 cells cultured in 12-well tissue culture plates as above were harvested by trypsin and EDTA treatment. After the cells were washed three times in 4° C saline, the brush border membranes from these cells were isolated following a previously described method (Schmitz et al., 1973; Pinto et al., 1983). Alkaline phosphatase levels in these preparations were determined by the method of Garen and Levinthal (1960) using *p*-nitrophenyl phosphate as a substrate. The assay was adapted for use in a 96-well EIA plate and the kinetic reaction was measured with the Biotek EL312e microplate reader. Total protein levels were determined with a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). One unit of enzyme activity was defined as the hydrolysis of 1 μmole of substrate per min under the described conditions.

Statistics. Values shown are the mean ± standard deviation of triplicate cultures of a single experiment which was representative of several experiments. Significant differences in mean values were determined with the Student's *t*-test except in Fig. 3 in which a regression analysis was performed.

Results

Induction of IL-6 secretion by Caco-2 cells. Culture of the Caco-2 cells with rhIL-1β or rhTNF-α with a protocol described in previous studies (McGee et al., 1992, 1993, 1995) resulted in the production of significantly elevated levels of IL-6 (Fig. 1). The effects of IL-1β and TNF-α stimulation on IL-6 secretion were both dose-dependent with a maximum stimulation occurring with 1 ng of IL-1β per ml or 50 ng of TNF-α per ml. Furthermore, an analysis of IL-6 mRNA expression by reverse transcriptase polymerase chain reaction showed elevated levels of IL-6 mRNA expression with IL-1β- or TNF-α-stimulated cells at 4 and 24 h (data not shown). Finally, stimulation of the Caco-2 cells with a combination of IL-1β plus TNF-α induced a synergistic enhancement of IL-6 secretion (unpublished results; also *see* Fig. 2) similar to that seen in previous studies with the IEC-6 cell line (McGee et al., 1995).

The specificity of the 7TD1 bioassay for detecting secreted human IL-6 was next tested. Aliquots of an IL-1β plus TNF-α-stimulated Caco-2 cell culture supernatant which was determined to contain 75 pg of IL-6 per ml were preincubated for 30 min with 100 μg/ml of either a neutralizing goat anti-human IL-6 antibody (R&D Systems) or normal goat IgG (Sigma) as a control. These supernatants were then added to the 7TD1 cells in the standard bioassay. As shown in Table 1, incubation of the supernatants with the neutralizing antibody resulted in the complete inhibition of 7TD1 cell proliferation, whereas the normal goat IgG control had no effect. A similar result was also seen when we used a second, separate IL-1β plus TNF-α-stimulated Caco-2 cell supernatant (data not shown). These results confirm that the 7TD1 cell bioassay was measuring IL-6 produced by the Caco-2 cells.

Effect of atmosphere CO_2 concentration on the capacity of Caco-2 cells to produce IL-6. A survey of the literature on studies of the Caco-2 cells revealed that both 5% or 10% CO_2 incubation atmospheres have been used in many studies. In an attempt to optimize the conditions for IL-6 secretion by the Caco-2 cells, we next tested the effect of incubator CO_2 concentration on IL-6 secretion. The culture medium was supplemented with 50 m*M* HEPES buffer to remove the possible effect of pH on the cultures. As expected, cells grown in 10% CO_2 yielded elevated levels of IL-6 secretion after stimulation with IL-1β, TNF-α, or both cytokines (Fig. 2). However, cells grown in 5% CO_2 produced significantly lower levels of IL-6 with stimulation by IL-1β, TNF-α, or both ($P < 0.05$ for each comparison) and TNF-α stimulation resulted in IL-6 levels which were not significantly different from unstimulated controls (Fig. 2). Although the

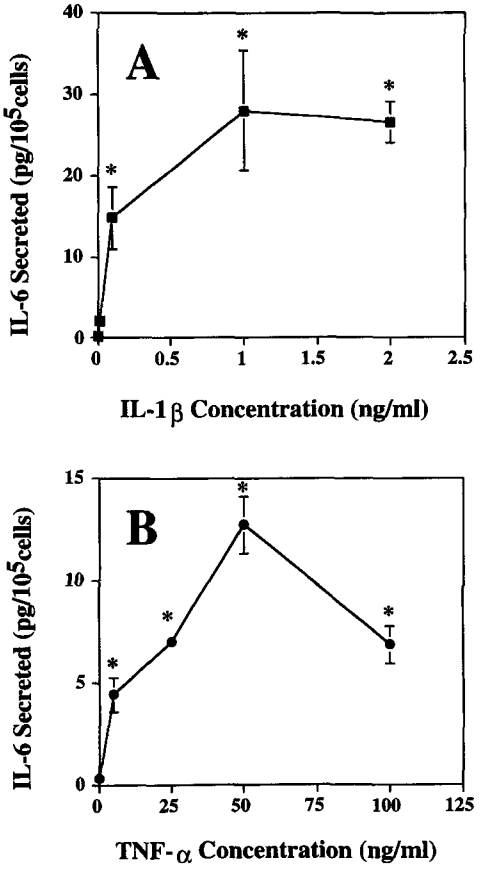

FIG. 1. Effect of IL-1β or TNF-α dose on IL-6 secretion by Caco-2 cells. The Caco-2 cells (2×10^5 cells/well in triplicate) were cultured for 2 d before addition of fresh medium containing (*A*) rhIL-1β or (*B*) rhTNF-α. After 2 d, the culture supernatants were collected for determination of IL-6 content and the adherent cells were removed by trypsin and EDTA treatment for counting. *Asterisks* indicate a significant difference from control cultures ($P < 0.05$). These figures are representative of three separate experiments each.

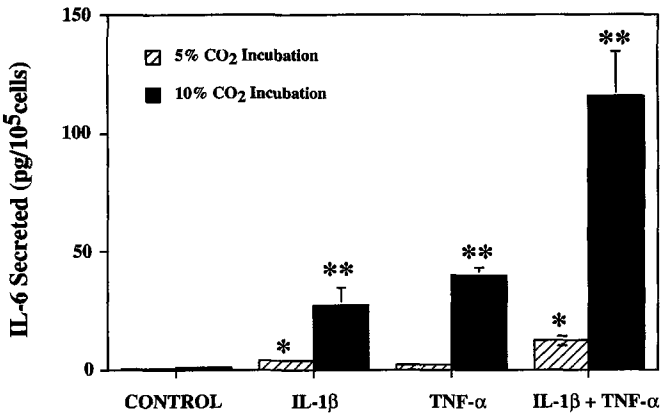

FIG. 2. The effect of incubator chamber CO_2 concentration on IL-6 secretion by Caco-2 cells. The Caco-2 cells (4×10^5 cells/well) were cultured in either a 5% or 10% CO_2 atmosphere for 7 d before we added rhIL-1β (1 ng/ml), rhTNF-α (50 ng/ml) or both in fresh 1% FCS-containing medium. Two d later the culture supernatants were collected for IL-6 determination and the cells harvested and counted. This figure is representative of three separate experiments. *Indicates a significant difference from unstimulated cultures in 5% CO_2 ($P < 0.05$). **Indicates a significant difference from unstimulated cultures in 10% CO_2 ($P < 0.05$).

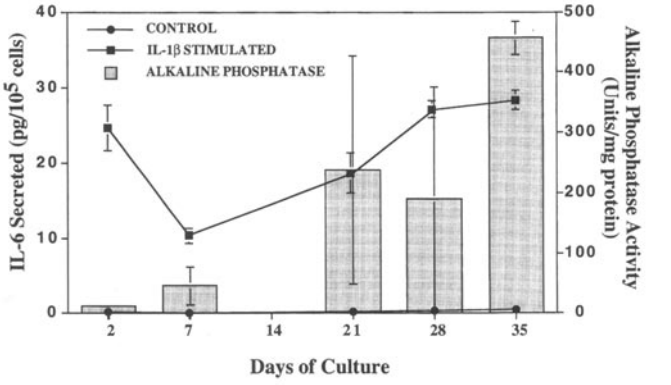

FIG. 3. Spontaneous differentiation of the Caco-2 cells had no effect on IL-6 secretion. The Caco-2 cells (2×10^5 cells/well) were cultured for the indicated number of d before the addition of fresh medium with or without 1 ng rhIL-1β per ml. Two d later the supernatants were collected and tested for IL-6 levels and the cells harvested and counted. Similar cultures were tested for brush border alkaline phosphatase levels as a measure of epithelial cell differentiation.

values shown in Fig. 2 were reported as per 10^5 cells, the actual final number of cells per well was not significantly different between cultures in either CO_2 condition, indicating that the difference was not due to a difference in total cell growth. These results suggest that the CO_2 concentration in the Caco-2 cell environment may be a significant factor in the capacity of these cells to produce IL-6 and should be taken into consideration in future studies on cytokine secretion by these cells.

Effect of Caco-2 cell differentiation on IL-6 secretion. Normal intestinal epithelial cells show a gradient of cellular differentiation across the crypt-to-villus axis with the more differentiated cells residing in the villus region. The migration of the IEC to the villus region is accompanied by many changes in cellular function as the cells differentiate. These include the expression of several enzymes associated with the brush borders, such as alkaline phosphatase, sucrose-isomaltase and aminopeptidase, and the appearance of these enzymes is often used as a marker for IEC differentiation (Louvard et al., 1992). The Caco-2 cells, when cultured to confluency, spontaneously begin to differentiate and show brush border microvilli with increasing levels of the brush border-associated enzymes (Pinto et al., 1983). Indeed, the cultures appear to go through three separate states of differentiation from homogeneously undifferentiated at subconfluence, to heterogeneously differentiated up to 20 d postconfluence, and homogeneously differentiated after approximately 30 d (Vachon and Beaulieu, 1992). This unique ability of the Caco-2 cells to spontaneously differentiate provides an excellent model system to determine the effect of IEC differentiation on the capacity of the cells to secrete IL-6. Therefore, cultures of the Caco-2 cells were initiated in 12-well culture plates as before and maintained in culture for up to 35 d. At Days 2, 7, 14, 21, 28, and 35, triplicate cultures were stimulated with fresh 1% FCS-containing medium with or without IL-1β. After 2 d, the culture supernatants were harvested for deter-

TABLE 1

IL-6 SECRETED FROM THE CACO-2 CELLS IS NEUTRALIZED BY A GOAT ANTI-HUMAN IL-6 ANTIBODY

Caco-2 supernatant[a]	Goat anti-human IL-6	Normal goat IgG	Optical density (570–650 nm)[b]
+	−	−	0.815 ± 0.03
+	+	−	0.002 ± 0.01
+	−	+	0.799 ± 0.05

[a]Aliquots of an IL-1β plus TNF-α stimulated Caco-2 cell culture supernatant that contained 75 pg of IL-6 per ml were preincubated 30 min with or without goat anti-human IL-6 (100 μg/ml) or normal goat IgG (100 μg/ml). The supernatants were then added to triplicate cultures of 7TD1 cells for the IL-6 bioassay.

[b]The proliferation of the 7TD1 cells was determined by the MTT colormetric proliferation assay.

mination of IL-6 levels and the cells counted as before. At the same time, similar cultures of Caco-2 cells were treated with fresh 1% FCS medium alone, and the cells were harvested for preparing brush borders and determining brush border enzyme activities.

The cultures of Caco-2 cells became confluent after 5 to 7 d of culture. As shown in Fig. 3, the alkaline phosphatase levels in the brush border membrane preparations increased with time, indicating that the Caco-2 cells differentiated as expected. However, the capacity of the cells at each interval to produce IL-6 when stimulated with IL-1β essentially did not change with the passage of time. Although the 7-d value for IL-6 secretion was lower than the values at 2 and 21 d, a regression analysis confirmed that there was no significant correlation between the alkaline phosphatase levels and IL-6 secretion ($P = 0.37$; $r^2 = 0.27$). This lack of correlation suggests that differentiation had no effect on the capacity of the cells to produce IL-6. Similar results were seen in two separate additional experiments.

Discussion

The IEC which line the intestine and colon are known to produce several cytokines which may be involved in cell-to-cell communications at the intestinal mucosa. In addition, the large number of these cells provides a potential mechanism to rapidly amplify any inflammatory signal with the appropriate release of proinflammatory cytokines by IEC such as IL-6. Because of the important role of IL-6 in the inflammatory response and B and T cell responses, our studies have focused on the regulation of IEC IL-6 secretion. Several studies have confirmed that normal intestinal and colonic IEC can produce IL-6 (Shirota et al., 1990; Jones et al., 1993; Woywodt et al., 1994; Jung et al., 1995; Kusugami et al., 1995; Panja et al., 1995), yet only a few aspects of the regulation of IL-6 secretion by IEC have been addressed (McGee et al., 1992, 1993, 1995; Jung et al., 1995; Panja et al., 1995). Further, many of our findings with the rat small intestinal epithelial cell line IEC-6 have not been confirmed for human IEC due to the difficulty of long term culture of isolated IEC and the lack of appropriate human IEC cell lines which secrete IL-6 similar to isolated IEC. Therefore, the first focus of this study was to identify a human IEC cell line which secretes IL-6 and characterize the secretion of this cytokine by these cells.

In this study, we found that the Caco-2 cell line can be induced by IL-1β or TNF-α to produce significant levels of IL-6 under the appropriate conditions. This capacity of the Caco-2 cells to produce IL-6 when stimulated by these cytokines was somewhat surprising, as previous reports have documented that neither mRNA for IL-6 nor IL-6 protein was detected in either unstimulated or stimulated Caco-2 cells (Eckmann et al., 1993; Jung et al., 1995). Still, the production of IL-6 by Caco-2 cells in the present study was confirmed by neutralizing experiments with a specific anti-human IL-6 antibody and experiments which showed an increase in IL-6 mRNA levels in stimulated cells. This discrepancy may have been due to different culturing conditions which we have found to be important for optimal IL-6 secretion (10% CO_2 incubation atmosphere as opposed to 5% CO_2). In addition, a recent report has shown that both the IEC-6 and Caco-2 cell lines produce significant levels of IL-6 when cultured with *Salmonella typhi* (Weinstein et al., 1997).

The Caco-2 cell line has recently been shown to be capable of secreting or producing mRNA for IL-8, TNF-α, TGF-β, and granulocyte monocyte-colony stimulating factor (Eckmann et al., 1993; Jung et al., 1995; McGee and McGhee, 1996) similar to normal human IEC. We now provide evidence that these cells also secrete IL-6 as do normal IEC. Taken together, these studies suggest that the Caco-2 cell line would prove to be an excellent model to study many aspects of normal IEC cytokine regulation which are impossible with isolated normal IEC due to culturing restraints.

We have now begun to characterize the regulation of IL-6 secretion by the Caco-2 cells. The Caco-2 cells were found to constitutively secrete only very low levels of IL-6, yet IL-1β or TNF-α stimulation greatly enhanced IL-6 secretion and IL-6 mRNA expression. Panja and coworkers (1995) have reported that IL-6 secretion by isolated human intestinal and colonic IEC could be enhanced by IL-1β stimulation but not by TNF-α. This discrepancy may be due to the greater than 200-fold spontaneous increase in IL-6 mRNA expression within the first 24 h in their cultures which could have masked an effect of TNF-α. This large spontaneous increase in IL-6 mRNA expression also indicates that their cells may have been activated during the isolation procedure and underscores the importance of using cultured cell lines for regulatory studies, as these cells normally exist in a low state of activation.

The capacity for the Caco-2 cell line to produce elevated levels of IL-6 with IL-1β or TNF-α stimulation and the synergistic effect of both of these cytokines on IL-6 secretion was similar to results from our previous study using the rat IEC-6 cell line (McGee et al., 1995), suggesting that the regulation of IL-6 secretion by human IEC may be similar to IEC from other species. It would be of interest to determine if Caco-2 cells also showed other IL-6 secretion characteristics previously shown with the IEC-6 cells such as the polarized secretion of IL-6 with basal IL-1β stimulation, but not TNF-α stimulation, when the cells were grown on microporous membranes in transwell cultures (Mascarenhas et al., 1996). Since the Caco-2 cells have been shown to polarize when cultured for greater than 14 d after confluence (Pinto et al., 1983), we expect that this cell line would provide an excellent model for these and other studies on polarized cytokine secretion by IEC. Also, the fact that both the Caco-2 colonic cell line and the IEC-6 small intestinal cell line showed similar stimulatory patterns for IL-6 secretion strongly suggests that IEC from intestine and colon may function in a similar manner. This capacity of the IEC to secrete IL-6 may then act as a mechanism to amplify any local IL-1β or TNF-α inflammatory signal from mucosal macrophages or TNF-α from panneth cells (Keshav et al., 1990) or other normal EC (Jung et al., 1995). The elevated IL-6 levels may then enhance local Ig secretion or T cell responses (Akira et al., 1993), induce the secretion of acute phase proteins by nearby IEC (Molmenti et al., 1993), or induce other inflammatory effects.

Finally, the Caco-2 cell line is perhaps the best model currently available to study the effects of intestinal IEC differentiation on cellular function. The IEC-6 cell line is an undifferentiated, crypt-like IEC line (Quaroni et al., 1979) and does not spontaneously differentiate in culture. Although IEC-6 cell differentiation has been induced by culture of the cells on a complex extracellular matrix (Carroll et al., 1988), few studies have used this cell line for differentiation studies. However, the Caco-2 cells spontaneously differentiate to mature villus-like cells after confluency (Pinto et al., 1983) and have been used in many studies of intestinal epithelial cell differentiation. Investigators using immunohistochemistry have suggested that both crypt and villus IEC can secrete IL-6 (Shirota et al., 1990; Jones et al., 1993), yet it is still unclear whether differentiation may affect the quantitative aspects of IEC IL-6 secretion. This is an important concept since many inflammatory diseases may result in the destruction of the mature villus epithelial cells. Culture of the Caco-2 cells under conditions which allowed differentiation showed no effect on the capacity of the cells to secrete IL-6, suggesting that differentiation had no effect on IL-6 secretion. Yet, since we did not determine the IL-6 secretion status of single cells, the possibility exists that a subpopulation of nondifferentiating cells may have been responsible for the IL-6 secretion and these cells remained undifferentiated throughout the 35-d culture period. However, Vachon and Beaulieu (1992) have shown that the Caco-2 cells cultured for approximately 30 d after confluence were homogeneously differentiated, with 91% of the cells expressing sucrase-isomaltase enzymes as characteristic of differentiated IEC, suggesting that this technique results in a uniformly differentiated culture. The simple but important finding that differentiation did not affect Caco-2 IL-6 secretion indicates that maturation is not necessary for optimal IL-6 secretion and therefore both immature crypt IEC and mature IEC may be fully capable of participating in an inflammatory response at the intestinal mucosa.

In closing, we have described several basic aspects of the secretion of IL-6 by the human colonic epithelial cell line, Caco-2. This cell line appears to function similar to normal human colonic epithelial cells and the rat IEC-6 small intestinal epithelial line with respect to IL-6 secretion. The Caco-2 cell line should prove to be a powerful tool for further studies on the regulation of cytokine secretion, including IL-6, by human IEC, and studies addressing some of these issues are now in progress with both the Caco-2 and the rat IEC-6 cell lines.

Acknowledgments

This work was supported by US PHS grant DK 44617.

References

Akira, S.; Taga, T.; Kishimoto, T. Interleukin-6 in biology and medicine. Adv. Immunol. 54:1–78; 1993.

Carroll, K. M.; Wong, T. T.; Drabik, D. L., et al. Differentiation of rat small intestinal epithelial cells by extracellular matrix. Am. J. Physiol. 254:G355–360; 1988.

Chen, T. R. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp. Cell Res. 104:255–262; 1977.

Eckmann, L.; Jung, H. C.; Schurer-Maly, C., et al. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. Gastroenterology 105:1689–1697; 1993.

Garen, H.; Levinthal, C. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. Biochim. Biophys. Acta 38:470–483; 1960.

Hedges, S.; Svensson, M.; Svanborg, C. Interleukin-6 response of epithelial cell lines to bacterial stimulation in vitro. Infect. Immun. 60:1295–1301; 1992.

Jones, S. C.; Trejdosiewicz, L. K.; Banks, R. E., et al. Expression of interleukin-6 by intestinal enterocytes. J. Clin. Pathol. 46:1097–1100; 1993.

Jung, H. C.; Eckmann, L.; Yang, S. K., et al. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J. Clin. Invest. 95:55–65; 1995.

Keshav, S.; Lawson, L.; Chung, L. P., et al. Tumor necrosis factor mRNA localized to panneth cells of normal murine intestinal epithelium by in situ hybridization. J. Exp. Med. 171:327–332; 1990.

Kusugami, K.; Fukatsu, A.; Tanimoto, M., et al. Elevation of interleukin-6 in inflammatory bowel disease is macrophage- and epithelial cell-dependent. Dig. Dis. Sci. 40:949–959; 1995.

Louvard, D.; Kedinger, M.; Hauri, H. P. The differentiating intestinal epithelial cell: establishment and maintenance of functions through interactions between cellular structures. Annu. Rev. Cell Biol. 8:157–195; 1992.

Mascarenhas, J. O.; Goodrich, M. E.; Eichelberger, H., et al. Polarized secretion of IL-6 by intestinal epithelial cells: differential effects of IL-1β and TNF-α. Immunol. Invest. 25:333–340; 1996.

McGee, D. W.; Beagley, K. W.; Aicher, W. K., et al. Transforming growth factor-β enhances interleukin-6 secretion by intestinal epithelial cells. Immunology 77:7–12; 1992.

McGee, D. W.; Beagley, K. W.; Aicher, W. K., et al. Transforming growth factor-β and IL-1β act in synergy to enhance IL-6 secretion by the intestinal epithelial cell line, IEC-6. J. Immunol. 151:970–978; 1993.

McGee, D. W.; Bamberg, T.; Vitkus, S. J. D., et al. A synergistic relationship between TNF-α, IL-1β, and TGF-β1 on IL-6 secretion by the IEC-6 intestinal epithelial cell line. Immunology 86:6–11; 1995.

McGee, D. W.; McGhee, J. R. Cytokine production by intestinal epithelial cells. In: Kaiserlian, D., ed. Antigen presentation by intestinal epithelial cells. Austin, TX: R. G. Landes; 1996:95–106.

Molmenti, E. P.; Ziambaras, T.; Perlmutter, D. H. Evidence for an acute phase response in human intestinal epithelial cells. J. Biol. Chem. 268:14116–14124; 1993.

Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J. Immunol. Methods 65:55–63; 1983.

Pang, G.; Buret, A.; O'Loughlin, E., et al. Immunologic, functional, and morphological characterization of three new human small intestinal epithelial cell lines. Gastroenterology 111:8–18; 1996.

Panja, A.; Siden, E.; Mayer, L. Synthesis and regulation of accessory/proinflammatory cytokines by intestinal epithelial cells. Clin. Exp. Immunol. 100:298–305; 1995.

Pinto, M.; Robine-Leon, S.; Appay, M., et al. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol. Cell 47:323–330; 1983.

Quaroni, A.; Wands, J.; Trelstad, R. L., et al. Epithelioid cell cultures from rat small intestine. J. Cell. Biol. 80:248–265; 1979.

Schmitz, J.; Preiser, H.; Maestracci, D., et al. Purification of the human intestinal brush border membrane. Biochim. Biophys. Acta 323:98–112; 1973.

Shirota, K.; LeDuy, L.; Yuan, S., et al. Interleukin-6 and its receptor are expressed in human intestinal epithelial cells. Virchows Arch. B Cell Pathol. 58:303–308; 1990.

Vachon, P. H.; Beaulieu, J. F. Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line. Gastroenterology 103:414–423; 1992.

Van Snick, J.; Cayphas, S.; Vink, A., et al. Purification and NH_2-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas. Proc. Natl. Acad. Sci. USA 83:9679–9683; 1986.

Weinstein, D. L.; O'Neill, B. L.; Metcalf, E. S. *Salmonella typhi* stimulation of human intestinal epithelial cells induces secretion of epithelial cell-derived interleukin-6. Infect. Immun. 65:395–404; 1997.

Woywodt, A.; Neustock, P.; Kruse, A., et al. Cytokine expression in intestinal mucosal biopsies. In situ hybridisation of the mRNA for interleukin-1β, interleukin-6 and tumour necrosis factor-α in inflammatory bowel disease. Eur. Cytokine Netw. 5:387–395; 1994.