Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1578–1582, March 1989
Cell Biology

Regulation of intestinal epithelial cell growth by transforming growth factor type β

(autocrine growth control/cell differentiation)

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Communicated by Robert W. Holley, December 5, 1988 (received for review October 4, 1988)

ABSTRACT **A nontransformed rat jejunal crypt cell line (IEC-6) expresses transforming growth factor type β1 (TGF-β1) mRNA, secretes latent ^{125}I-labeled TGF-β1 competing activity into culture medium, and binds ^{125}I-labeled TGF-β1 to specific, high-affinity (K_d = 3.7 pM) cell surface receptors. IEC-6 cell growth is markedly inhibited by TGF-β1 and TGF-β2 with half-maximal inhibition occurring between 0.1 and 1.0 ng of TGF-β1 per ml. TGF-β1-mediated growth inhibition is not associated with the appearance of biochemical markers of enterocyte differentiation such as alkaline phosphatase expression and sucrase activity. TGF-β1 (10 ng/ml) increases steady-state levels of its own mRNA expression within 8 hr of treatment of rapidly growing IEC-6 cells. In freshly isolated rat jejunal enterocytes that are sequentially eluted from the crypt villus axis, TGF-β1 mRNA expression is most abundant in terminally differentiated villus tip cells and least abundant in the less differentiated, mitotically active crypt cells. We conclude that TGF-β1 is an autoregulated growth inhibitor in IEC-6 cells that potentially functions in an autocrine manner. In the rat jejunal epithelium, TGF-β1 expression is most prominently localized to the villus tip—i.e., the region of the crypt villus unit that is characterized by the terminally differentiated phenotype. These data suggest that TGF-β1 may function in coordination of the rapid cell turnover typical for the intestinal epithelium.**

Epithelial cells situated along the intestinal crypt villus axis represent a dynamic continuum of enterocyte structure and function. Cells in the crypt are rapidly proliferating and less differentiated, whereas cells at the villus tip are terminally differentiated and possess specialized absorptive and digestive functions. Continuous extrusion or exfoliation of cells at the villus tip is usually counterbalanced by ongoing proliferation in the crypt such that the net intestinal epithelial mass remains relatively constant. Turnover of the entire crypt villus epithelial cell population occurs every 24–72 hr, one of the most rapid turnover rates of any tissue in the body (1). A larger number of intraluminal and systemic factors purportedly regulate normal growth of the gastrointestinal tract (reviewed in ref. 2); nonetheless, fundamental questions relevant to development, growth, differentiation, and adaptation of the intestinal epithelium remain unanswered and unexplored. Understanding the mechanisms for normal growth control will undoubtedly facilitate comprehension of those mechanisms responsible for the aberrant or adaptive cell kinetics seen during lactation (3), gastroenteritis (4), streptozotocin-diabetes (5), short bowel syndrome (6), and malnutrition (7).

Recent studies have suggested that growth stimulatory autocrine polypeptides such as transforming growth factor type α (TGF-α) and growth inhibitory autocrine polypeptides such as those belonging to the TGF-β gene family may play an integral role in the control of normal epithelial cell growth. Barrandon and Green (8) noted that TGF-α stimulated human keratinocyte proliferation *in vitro*. Coffey *et al.* (9) demonstrated that primary cultures of normal human keratinocytes synthesized TGF-α; analysis of human skin biopsies showed the presence of TGF-α mRNA and protein in the stratified epidermis (10). Other studies showed that TGF-β reversibly inhibited growth of human keratinocytes and BALB/MK cells (mouse keratinocyte) and that TGF-β-mediated growth inhibition of BALB/MK cells occurs as a result of selective down-regulation of at least two genes that are induced early in response to epidermal growth factor (EGF)—i.e., c-*myc* and KC (11, 12). Further evidence for an *in vivo* regulatory role for TGF-β in epithelial homeostasis has been shown in mouse skin in which TGF-β mRNA was induced in the suprabasal, differentiating epidermis by the tumor promoter phorbol 12-myristate 13-acetate (13).

Based on these observations, requisite elements for stimulatory and inhibitory autocrine growth control mechanisms appear to exist in normal skin. Inasmuch as the skin and the gastrointestinal epithelium are self-renewing epithelia, and aberrant autocrine growth control pathways have been described in several colorectal carcinoma cell lines (14), we have hypothesized that TGF-β is an integral physiologic modulator of normal small intestinal epithelial growth and differentiation. In the present communication, evidence is presented in support of a TGF-β autocrine axis in cultured normal intestinal epithelial cells and differential expression of TGF-β1 mRNA along the rat jejunal crypt villus axis.

METHODS

Cell Culture. Nontransformed rat jejunal crypt cells (IEC-6, passage 17) were obtained from the American Type Culture Collection. IEC-6 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% dialyzed fetal bovine serum (GIBCO) and 5 μg of insulin per ml. No antibiotics were used. AKR-2B (clone 84A) were grown in McCoy's medium 5A supplemented with 5% fetal bovine serum.

Elution (Fractionation) of Rat Enterocytes. The technique was slightly modified from Weiser's original method (15). In selected experiments, three rats were given 100 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine by intraperitoneal injection 3 hr prior to euthanasia. A 12-cm segment of the proximal jejunum was removed, rinsed in 165 mM NaCl with 1 mM dithiothreitol, and carefully everted over a tightly fitting glass rod. The everted segment was then immersed in buffer A (1.5 mM KCl/96 mM NaCl/27 mM sodium citrate/8 mM KH_2PO_4/5.6 mM Na_2PO_4, pH 7.4) at 37°C that was preoxygenated with 100% O_2. After 10 min in a shaking water bath at 37°C, the

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Abbreviations: TGF, transforming growth factor; EGF, epidermal growth factor.

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everted segment was transferred to preoxygenated buffer B (109 mM NaCl/2.4 mM KCl/1.5 mM KH_2PO_4/4.3 mM Na_2HPO_4/0.5 mM EDTA/10 mM glucose/5 mM glutamine/0.5 mM dithiothreitol) for the first of four 10-min collection periods. The fifth collection interval was 20 min. During incubation in buffer B, the everted segments were shaken at 75 oscillations per min in a 37°C water bath. Greater than 95% of cells in the last fraction excluded trypan blue. Light microscopic examination of the epithelial cell fractions showed no contamination by cells originating in the lamina propria.

Isolation of Poly(A) RNA from IEC-6 Cells. Total cellular RNA was extracted by the method of Schwab *et al.* (16). Oligo(dT)-selected RNA was separated by electrophoresis in a 1% agarose/formaldehyde gel, and Northern blotting was performed as described (17). Isolated cDNA insert probes were radiolabeled by random primer extension using [^{32}P]-dCTP and [^{32}P]dATP (18). The TGF-β1 probe is a 974-base-pair (bp) *Sma* I insert of a mouse cDNA clone derived from plasmid pBR322. The plasmid contains the entire open reading frame and 440 bp of untranslated cDNA (19). A rat cyclophilin cDNA insert (1B15) is used as a marker for constitutive gene expression (20). The alkaline phosphatase probe is a 330-bp *Eco*RI insert of a rat placental alkaline phosphatase cDNA (21) and was a generous gift from David Alpers. The TGF-β2 probe is an *Eco*RI–*Hpa* I 2.2-kb insert isolated from a human prostatic adenocarcinoma cell line (22). The TGF-β3 probe is an 880-bp *Bgl* II human cDNA clone (23). Hybridizations were performed in 5× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), 50% deionized formamide, 1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 0.1% sodium dodecyl sulfate (SDS), 50 μg of poly(A) per ml, and 250 μg of salmon sperm DNA per ml at 43°C for 18 hr. Posthybridization washes were done in 0.1× SSC, 0.1% SDS, and 1 mM EDTA at 43°C.

Isolation of Poly(A) RNA from Rat Enterocytes. Total cellular RNA was isolated from freshly fractionated rat enterocytes by a modification of the method reported by Chirgwin *et al.* (24). Briefly, after elution, each enterocyte fraction was centrifuged at 1000 × *g* for 5 min. The pellet was immersed in 8 ml of ice-cold 4 M guanidinium isothiocyanate containing 1% 2-mercaptoethanol and immediately homogenized for 15 sec in a Tekmar homogenizer at 75% maximal speed. The homogenate was layered onto a 5.7 M CsCl cushion and ultracentrifuged at 179,000 × *g* for 15 hr at 22°C. The resulting pellet was resuspended in sterile water with 0.01% SDS and 0.5 M NaCl and extracted with salt-saturated phenol and chloroform. RNA was precipitated in 2.5 vol of 95% ethanol. If all RNA fractions were judged to be intact by agarose gel electrophoresis, the RNA was oligo(dT)-selected and used for Northern blot analysis as described above.

Growth Assays. IEC-6 cells were plated in tissue culture plates (35 mm^2 per well) containing DMEM with 5% fetal bovine serum and 5 μg of insulin per ml. When the cells reached 75% confluence, the medium was changed to serum-free DMEM for 24 hr. Growth factors were then reintroduced and cells were counted 48 hr later using a hemocytometer. Cell counts were done in triplicate.

Measurement of TGF-β1 Competing Activity in IEC-6-Conditioned Medium. IEC-6 cells were grown to confluence in 150-cm^2 flasks (Falcon). After three washes in buffered saline solution A (25), the medium was changed to serum-free DMEM. The first 24-hr collection was discarded. The next 48-hr collection was removed, centrifuged briefly to remove cellular debris, and then treated with 2 mM phenylmethylsulfonyl fluoride. Aliquots were acid treated by addition of 1.0 M HCl to pH 2.0 for 30 min and then reneutralized by the addition of 1.0 M NaOH to pH 7.3. Aliquots were then assayed for ^{125}I-labeled TGF-β1 competing activity in a previously described TGF-β1 radioreceptor assay (14).

TGF-β1 Binding to IEC-6 Cells. Nearly confluent IEC-6 cells in monolayer were washed in binding buffer (128 mM NaCl/5 mM KCl/1.2 mM $CaCl_2$/1.2 mM $MgCl_2$/50 mM Hepes, pH 7.4/2 mg of bovine serum albumin per ml) for 1 hr at room temperature. Binding assays were then performed in binding buffer at 4°C for 120 min with the indicated concentrations of ^{125}I-labeled TGF-β1 and unlabeled TGF-β1. Binding was terminated by gently washing the monolayer three times with ice-cold binding buffer. Bound radioactivity was measured by solubilizing membranes in 1% Triton X-100 in phosphate-buffered saline and counting radioactivity in a γ-counter. TGF-β1 was labeled with ^{125}I by using a previously described chloramine-T method (26).

Other Assays. Alkaline phosphatase activity was determined as described (15). [^{3}H]Thymidine incorporation into DNA was measured by isolation of the DNA band from CsCl gradients used for RNA purification and counting aliquots of trichloroacetic acid-precipitable material for radioactivity. DNA was quantified by the method described by Burton (27), and protein was quantified by the method of Lowry *et al.* (28). Sucrase activity was determined by the method of Dahlquist (29).

RESULTS

TGF-β1 mRNA Expression in Intestinal Epithelial Cells. Poly(A) RNA samples isolated from confluent IEC-6 cells, rat jejunal epithelial cells, and HT-1080 cells were analyzed by Northern hybridization for TGF-β1 mRNA. IEC-6 and normal rat jejunal epithelial cells showed a 2.5-kilobase (kb) transcript that is equivalent in size to that seen in HT-1080, a human fibrosarcoma cell line that is known to express TGF-β1 (30) (Fig. 1).

Little is known regarding factors that regulate expression of the TGF-β1 gene. Recently, several investigators described a phenomenon of "autoinduction" of polypeptide growth factor mRNA expression and protein secretion—that is, enhanced expression of the growth factor in response to exposure of cells to that same factor (10, 31, 32). When rapidly growing IEC-6 cells were exposed to 10 ng of TGF-β1 per ml, TGF-β1 mRNA expression increased to a level 4-fold above control cells within 8 hr and maintained this level of expression for at least 24 hr (Fig. 2). Levels of 1B15 RNA remained unchanged. It is postulated that autoinduction of TGF-β1 expression represents a mechanism for signal amplification that functions to promote a sustained response to physiologic or pathophysiologic stimuli.

TGF-β1 Production by IEC-6 Cells. The presence of TGF-β-like competing activity in DMEM conditioned by IEC-6 cells for 48 hr was assessed by competitive radioreceptor assay. AKR-2b (clone 84A) cells were used in the binding assay as described (14). The unconcentrated conditioned medium was treated with 2 mM phenylmethylsulfonyl fluoride, acidified to pH 2 with HCl for 30 min, and reneutralized with NaOH. This process results in activation of latent TGF-β (33, 34). As shown in Fig. 3*A*, increasing concentra-

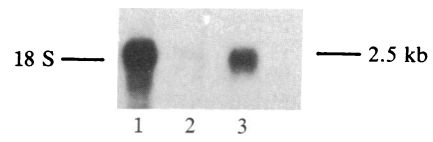

FIG. 1. Expression of TGF-β1 in intestinal epithelial cells. Five micrograms of poly(A) RNA isolated from IEC-6 cells (lane 1) and rat jejunum (lane 2) and 1 μg of poly(A) RNA from HT-1080 cells (positive control, lane 3) were analyzed by Northern blot. Nitrocellulose filters were hybridized with [^{32}P]dCTP- and [^{32}P]dATP-labeled TGF-β1 cDNA.

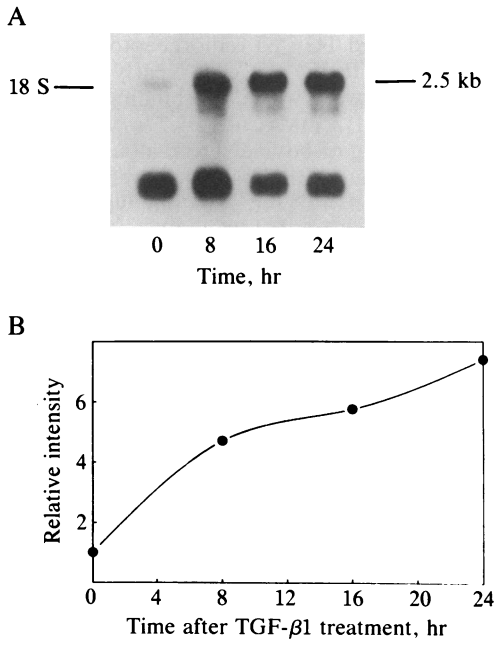

FIG. 2. Autoinduction of TGF-β1 expression in IEC-6 cells. Northern blot analysis of poly(A) RNA (4 μg per lane) isolated from IEC-6 cells. Cells were allowed to achieve a confluency of ≈75% in 150-cm² tissue culture flasks containing 5% DMEM and 5 μg of insulin per ml. TGF-β1 (10 ng/ml) was added at time 0 and poly(A) RNA was isolated at the times shown. Blots were probed with TGF-β1 and 1B15 probes (*A*). The autoradiograms were scanned by laser densitometry; the TGF-β1 signal intensity was normalized to 1B15 and expressed as relative intensity (*B*).

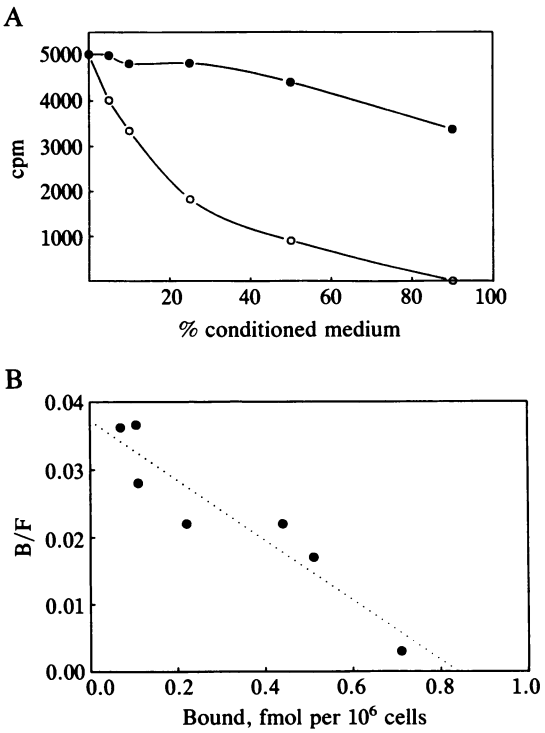

FIG. 3. (*A*) Production of TGF-β-like material by IEC-6 cells. IEC-6-conditioned medium was collected and treated. Increasing volumes of 48-hr conditioned medium were added to binding buffer in the presence of tracer concentrations of ^{125}I-labeled TGF-β1. Shown is specific ^{125}I-labeled TGF-β1 binding and the percentage of the binding buffer represented by conditioned medium. ●, Neutral; ○, acid treated. (*B*) Specific, saturable ^{125}I-labeled TGF-β1 binding to IEC-6 monolayers. Cells were incubated with tracer concentrations of ^{125}I-labeled TGF-β1 at 4°C. Specific binding was analyzed by Scatchard plot. Binding parameters were a K_d of 3.7 pM and 3100 TGF-β1 receptors per cell. B/F, bound/free.

tions of acid-activated, conditioned medium effectively competed for specific ^{125}I-labeled TGF-β1 binding sites on AKR-2b (clone 84A) cells, with complete inhibition occurring at 90% conditioned medium. Neutral conditioned medium competed poorly. These data indicate that TGF-β is synthesized and secreted in an acid-activatable, latent form by IEC-6 cells.

TGF-β1 Binding to IEC-6 Cells. Preliminary assays showed that ^{125}I-labeled TGF-β1 binding to IEC-6 cells reached a maximum after 2 hr of incubation at 22°C, whereas at 4°C binding reached a plateau at 2 hr and maintained this level for at least 4 hr (data not shown). For purposes of Scatchard analysis, equilibrium binding was assumed to occur after 2 hr of incubation of 4°C. Specific IEC-6 cell surface receptors for TGF-β1 were characterized by incubation of cells with ^{125}I-labeled TGF-β1 concentrations between 2 and 400 pM. Nonspecific binding was determined by incubation with a 200-fold excess of unlabeled TGF-β1. Scatchard analysis of specific TGF-β1 binding showed a single high-affinity binding site (K_d = 3.7 pM) and a receptor density of 3100 per cell (Fig. 3*B*).

Effect of TGF-β1 and TGF-β2 on Proliferation of IEC-6. Serum and serum with added EGF (10 ng/ml) stimulated IEC-6 cell proliferation. TGF-β1 and TGF-β2 inhibited serum-stimulated proliferation of IEC-6 cells, an effect that was first detected in growth assays 48 hr after treatment of subconfluent monolayers with 10 ng of TGF-β1 per ml (Fig. 4*A*). Equimolar concentrations of TGF-β1 and TGF-β2 were comparable in their inhibition of IEC-6 cell proliferation (data not shown). Half-maximal inhibition of IEC-6 proliferation occurred at a TGF-β1 concentration between 0.1 ng/ml and 1.0 ng/ml (0.4 and 4 pM) (Fig. 4*B*). To test the hypothesis that TGF-β1 inhibits proliferation of IEC-6 cells by induction of a terminal differentiation pathway, sucrase and alkaline phosphatase assays were performed on cell homogenates prepared from rapidly growing as well as confluent monolayers treated for 24–48 hr with 10 ng of TGF-β1 per ml. In a separate study, alkaline phosphatase mRNA expression was monitored for 24 hr following treatment with TGF-β1 at 10 ng/ml of rapidly growing and confluent IEC-6 monolayers. In each instance, no induction of these enzymatic markers of intestinal epithelial cell differentiation was detected (data not shown).

Expression of Rat Jejunal TGF-β1 mRNA in Enterocytes Eluted from the Crypt Villus Axis. To assess the biological relevance of TGF-β expression in IEC-6 cells, poly(A) RNA was isolated from jejunal enterocytes serially eluted from the crypt villus axis. These samples were analyzed by Northern hybridization for the presence of TGF-β1, TGF-β2, and TGF-β3 mRNA. TGF-β2 and TGF-β3 transcripts were not detectable (data not shown) under conditions that readily yielded TGF-β1 hybridization (Fig. 5*A*). Fraction 1 (villus tip cells) yielded the most abundant levels of the 2.5-kb TGF-β1 transcript, whereas fraction 5 consistently yielded little or no TGF-β1 mRNA. In contrast, levels of 1B15 mRNA, a constitutively expressed transcript (20), were relatively equally detected in all fractions. Fig. 5*B* shows the relative expression of TGF-β1 from a single, representative RNA preparation. Also shown is alkaline phosphatase activity, a marker for the differentiated enterocyte, and incorporation of intraperitoneally injected [^{3}H]thymidine into enterocyte DNA, a measure of enterocyte proliferative potential. TGF-β1 expression increased in conjunction with increasing alkaline phosphatase specific activity, whereas [^{3}H]thymidine incorporation decreased, indicating an association with the differentiated phenotype and an inverse association with

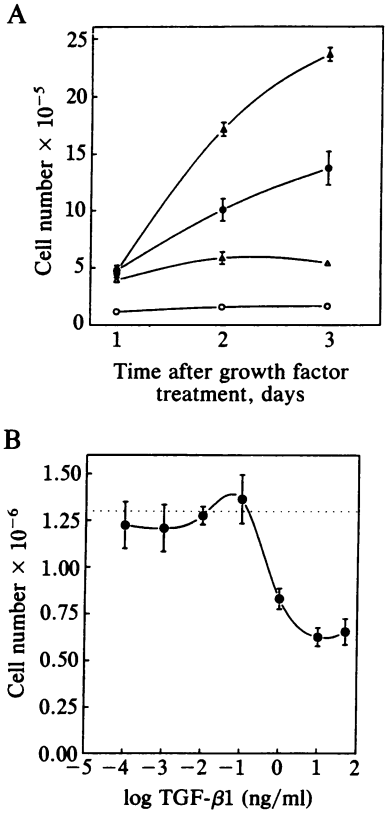

FIG. 4. (*A*) TGF-β1 inhibition of IEC-6 cell growth. IEC-6 monolayers were grown to 75% confluence, serum deprived for 24 hr, and then restimulated under the conditions shown. The cells were trypsinized and counted in a hemocytometer 1, 2, and 3 days after restimulation. Each point represents the mean ± SEM for triplicate counts. ○, DMEM alone; △, 5% serum + TGF-β1 at 10 ng/ml; ●, 5% serum; ▲, 5% serum + EGF at 10 ng/ml. (*B*) Inhibition of IEC-6 cell growth is TGF-β1 concentration dependent. Cells were treated as described in *A*. Monolayers were restimulated with 5% fetal bovine serum alone (dotted line) or 5% fetal bovine serum plus the indicated concentration of TGF-β1 (closed circles). Cells were counted 48 hr later and results are plotted as mean ± SEM for triplicate counts.

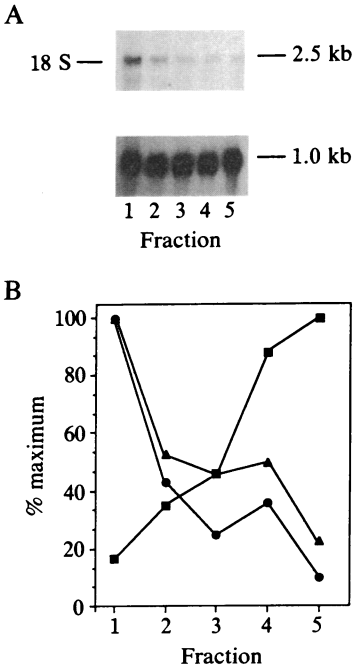

FIG. 5. (*A*) Differential expression of TGF-β1 RNA in freshly isolated rat jejunal enterocytes. Each lane was loaded with 5 μg of poly(A) RNA isolated from rat enterocytes. After Northern blotting, the nitrocellulose filter was exposed to [^{32}P]dCTP- and [^{32}P]dATP-labeled TGF-β1 and 1B15 cDNA probes and autoradiograms were prepared. Lane 1 shows hybridization with RNA isolated from a villus tip fraction and subsequent lanes represent fractions progressively deeper toward the crypt (see *B*). The larger (2.5 kb) transcript is TGF-β1 and the smaller (1.0 kb) is 1B15. (*B*) Relative expression of TGF-β1 RNA, alkaline phosphatase activity, and [^{3}H]thymidine incorporation into enterocyte DNA. TGF-β1 expression (▲) (*A*) along the crypt villus axis was quantitated by laser densitometry and normalized to expression of 1B15. The data are plotted as % maximal expression. Alkaline phosphatase activity (●) (μmol/min per mg of protein) and cpm of [^{3}H]thymidine (■) incorporated per μg of DNA are similarly displayed as a % of maximal activity. Fraction 1 is the first eluted enterocyte fraction and fraction 5 is the last.

enterocyte proliferation. Identical results were found in two additional, separate experiments.

DISCUSSION

The autocrine hypothesis was first developed in conjunction with the discovery of TGF-α in an attempt to explain the uncontrolled cellular proliferation characteristically observed in neoplastic cells (35). There now exists a large body of circumstantial and direct evidence in support of the autocrine hypothesis as it relates to neoplasia (36, 37). Recent observations have suggested that the concept of autocrine growth control should be broadened to encompass a role for autocrine growth factors in the control of normal epithelial cell growth. The preponderance of this work has been done using skin-derived epithelial systems and is strongly supportive of a role for TGF-α and TGF-β in normal growth control (9, 10) in wound healing (38), and in diseases of the skin such as psoriasis (39).

Although a great deal is known about the role of dietary influences and systemic hormones in the control of proliferation and differentiation of the gastrointestinal tract epithelium, basic questions remain concerning modulation of the rapid enterocyte turnover rate and the signaling mechanisms responsible for differentiation. Based on the aforementioned studies using mouse and human keratinocytes and biopsies of normal and diseased skin, growth stimulatory and growth inhibitory autocrine growth factors are potential candidates as modulators of intestinal epithelial growth. Of particular interest is TGF-β, a M_r 25,000 homodimer that potently inhibits normal epithelial cell growth and modulates differentiation in primary human bronchial epithelial cells (40), osteoblasts (41), adrenocortical cells (42), and myoblasts (43), among others. Podolsky and coworkers (44) recently reported that TGF-β inhibits growth of the jejunal epithelial crypt cell line IEC-6 and that these cells express sucrase activity within 18 hr of exposure to TGF-β. On the basis of these observations, the present study was designed to examine IEC-6 cells for the requisite elements of a growth inhibitory autocrine loop and to explore the potential relevance to *in vivo* gastrointestinal growth.

Currently three molecular forms of TGF-β have been identified: TGF-β1 (45), TGF-β2 (polyergin) (46), and TGF-β3 (47). TGF-β1 and TGF-β2 proteins have been purified; however, few biological differences are recognized. Consequently, the majority of data reported herein were obtained using porcine-derived TGF-β1 and a mouse TGF-β1 cDNA probe. Northern analysis of poly(A) RNA isolated from IEC-6 cells and a population of freshly isolated rat enterocytes showed the characteristic 2.5-kb TGF-β1 transcript. The presence of secreted TGF-β-like protein in serum-free IEC-6-conditioned medium was identified by radioreceptor assay. The interaction of TGF-β1 and TGF-β2 with specific cell surface membrane receptors has been extensively characterized in fibroblastic cells and appears to be a complex

phenomenon involving three ligands (TGFs β1, β2, and presumably β3) binding to three structurally distinct receptors (M_r 65,000, M_r 85,000–95,000, and a M_r 560,000–600,000 receptor complex) with varying affinities (48–50). Thus, demonstration of ^{125}I-labeled TGF-β1 competing activity by radioreceptor assay does not justify concluding that the competing material is TGF-β1 alone. Moreover, ligand–receptor cross-linking studies are required to identify TGF-β receptor types I–III. Scatchard analysis of ^{125}I-labeled TGF-β1 binding to IEC-6 cells revealed a single class of high-affinity receptors (K_d = 3.7 pM) and 3100 receptors per cell. These results are in close agreement with those obtained for other nontransformed epithelial cell lines such as BALB/MK (mouse keratinocytes) (K_d = 11.0 pM and 7000 receptors per cell; N. J. Sipes, personal communication).

TGF-β1 and TGF-β2 were potent inhibitors of IEC-6 growth, with half-maximal inhibition occurring at a TGF-β1 concentration between 0.1 ng/ml and 1.0 ng/ml. Previous work has suggested that TGF-β inhibits IEC-6 cell growth by induction of a terminal differentiation pathway (42). Under our experimental conditions, these observations could not be duplicated. Exposure to TGF-β1 (10 ng/ml) for up to 72 hr did not induce sucrase or alkaline phosphatase activity in quiescent or rapidly growing cells. Northern analysis of similarly treated IEC-6 poly(A) RNA using a rat placental alkaline phosphatase cDNA probe failed to reveal alkaline phosphatase transcripts, whereas freshly isolated rat enterocyte mRNA yielded an abundant signal (data not shown). The effects of TGF-β on cellular differentiation are diverse and include such variable effects as down-regulation of the differentiated phenotype in myoblasts (43) and rapid induction of morphologic and biochemical features of differentiation in human bronchial epithelial cells and osteoblasts (40, 41).

The potential relevance of the putative TGF-β autocrine loop described for IEC-6 cells to *in vivo* intestinal growth and differentiation was investigated by examination of enterocyte TGF-β1, TGF-β2, and TGF-β3 RNA expression as a function of enterocyte position along the crypt villus axis. There was no evidence for TGF-β2 and TGF-β3 mRNA production by isolated rat jejunal enterocytes. TGF-β1 expression was greatest in cells isolated from the villus tip—i.e., the portion of the crypt villus unit characterized by the absence of mitotic activity and the presence of mature, differentiated enterocyte function. By contrast, TGF-β1 expression was lowest in the proliferative zone (crypt), where differentiated function is not found. These observations suggest that TGF-β1 may function to arrest growth as the enterocyte leaves the proliferative zone and to maintain the terminally differentiated state. These findings are analogous to the demonstration of phorbol 12-myristate 13-acetate-induced TGF-β mRNA only in epidermal cells that have been committed to a terminal differentiation pathway (13). Although a direct role for TGF-β1 in enterocyte differentiation could not be shown using IEC-6 cells, it is plausible that TGF-β1 may function in concert with other stimuli *in vivo* to effect entry into a differentiation pathway.

This research was supported by Biomedical Research Support Grant RR05424, National Cancer Institute Grant CA46413, the Veterans Administration, and an American Gastroenterological Association/Pharmacia Scholar Award.

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