Growth Hormone Inhibits Apoptosis in Human Colonic Cancer Cell Lines: Antagonistic Effects of Peroxisome Proliferator Activated Receptor-γ Ligands

FAUSTO BOGAZZI, FEDERICA ULTIMIERI, FRANCESCO RAGGI, DANIA RUSSO, RENATO VANACORE, CHIARA GUIDA, SANDRA BROGIONI, CHIARA COSCI, MAURIZIO GASPERI, LUIGI BARTALENA, AND ENIO MARTINO

Department of Endocrinology and Metabolism (F.B., F.U., F.R., D.R., S.B., C.C., M.G., E.M.) and Transfusional Unit (R.V., C.G.), University of Pisa, 56124 Pisa, Italy; and Division of Endocrinology (L.B.), University of Insubria, 21100 Varese, Italy

GH has antiapoptotic effects on several cells. However, the antiapoptotic mechanisms of GH on colonic mucosa cells are not completely understood. Peroxisome proliferator activated receptor-γ (PPARγ) activation enhances apoptosis, and a link between GH and PPARγ in the colonic epithelium of acromegalic patients has been suggested. We investigated the effects of GH and of PPARγ ligands on apoptosis in colonic cancer cell lines. Colonic cells showed specific binding sites for GH, and after exposure to 0.05-50 nM GH, their apoptosis reduced by 45%. The antiapoptotic effect was due to either GH directly or GH-dependent local production of IGF-1. A 55–85% reduction of PPARγ expression was observed in GH-treated cells, compared with controls (P < 0.05). However, treatment of the cells with 1–50 μM ciglitazone (cig), induced apoptosis and reverted the antiapoptotic effects of GH by increasing the programmed cell death up to 3.5-fold at 30 min and up to 1.7-fold at 24 h. Expression of Bcl-2 and TNF-related apoptosis-induced ligand was not affected by either GH or cig treatment, whereas GH reduced the expression of Bax, which was increased by cig treatment. In addition, GH increased the expression of signal transducer and activator of transcription 5b, which might be involved in the down-regulation of PPARγ expression. In conclusion, GH may exert a direct antiapoptotic effect on colonic cells, through an increased expression of signal transducer and activator of transcription 5b and a reduction of Bax and PPARγ. The reduced GH-dependent apoptosis can be overcome by PPARγ ligands, which might be useful chemopreventive agents in acromegalic patients, who have an increased colonic polyps prevalence. (Endocrinology 145: 3353–3362, 2004)

GH AND OTHER growth factors have been shown to regulate the programmed cell death in several types of cells (1–6). GH increases the expression of homeo box A1, which in turn is involved in the abrogation of the apoptotic response of mammary carcinoma cells to doxorubicin (1); in addition, rats treated with GH have a reduced apoptosis of intestinal cells during the early course of acute necrotizing pancreatitis (2). Autocrine GH-induced repression of apoptosis-promoting proteins, in mammary carcinoma cells, might be linked to tumor progression (3). GH promotes cell cycle progression of lymphoid cells and prevents their apoptosis, mainly through the phosphorylation of Akt pathway and the transcription factor nuclear factor κB (NF-κB); by these molecular mechanisms, GH might play a role in regulating apoptosis, proliferation, and eventually neoplastic transformation of immune cells (4).

In bovine embryos GH did not affect expression of B-cell

Abbreviations: 7-AAD, 7-Actinomycin D; Ab, antibody; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CAT, chloramphenicol acetyl transferase; cig, ciglitazone; DCA, deoxycholic acid; FITC, fluorescein isothiocyanate; GHR, GH receptor; IGFI-IR, IGF-I receptor; NF-κB, nuclear factor κB, subunit I; PI, propidium iodide; PPAR, peroxisome proliferator activated receptor; PPRE, PPAR response element; ros, rosiglitazone; STAT, signal transducer and activator of transcription; TRS, Tris-buffered saline; TRAIL, TNF-related apoptosis-induced ligand; TZD, thiazolidinedione.

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at ion (14, 15). It is a functional receptor for the thiazolidinedione anti-diabetic drugs and may function as a tumor suppressor gene (16, 17); its activation has been reported to induce differentiation of liposarcoma (18), prostate cancer (19), or several transformed cells (14–20).

PPARγ expression increases during differentiation of colonic epithelial cells (21, 22), and its activation is associated with growth inhibition and increased levels of markers of cellular differentiation in cultured colon cancer cells (14).

In human and rat glioma cells, PPARγ activation induces apoptosis associated with a transient up-regulation of Bax and Bcl-2 antagonist of cell death protein levels (23). The troglitazone-induced apoptosis of human papillary thyroid carcinoma cells was associated with enhanced expression of c-myc, whereas expression of Bcl-2 and Bax proteins was unaffected (24). In colonic cancer HT-29 cell line, activation of PPARγ by troglitazone increased cell death associated with down-regulation of c-myc and up-regulation of c-jun and growth arrest and DNA damage-inducible gene 153 (25). In addition, in the colonic cancer cell line Caco2, PPARγ activation reduced cytokine gene expression inhibiting the NF-κB pathway (26).

The aim of the study was to examine the role of GH and PPARγ in the regulation of apoptosis in colonic epithelial cells.

**Materials and Methods**

**Cell culture and cell preparation**

Caco2, HT29, and NIH3T3 cell lines were obtained from Data Bank (Genoa, Italy) and cultured in DMEM, 20% (10% for NIH3T3 and HT29) fetal bovine serum, 1% nonessential amino acids (Caco2 cells only), and 2 mm glucose and penicillin-streptomycin solution in a humidified 5% CO₂ atmosphere at 37 C. Under starvation conditions, cells were extensively washed in PBS and then incubated for 12 h in serum-free medium.

To study the effect of GH or PPARγ activation on apoptosis, starved cells were cultured in the absence or presence of 0.05–50 nm human recombinant GH (Sigma-Aldrich, Milan, Italy), 0.1–100 µm cigitazone (cig) (Alexis Biochemicals, San Diego, USA), alone or in combination. Some experiments were performed using 1–100 µm rosiglitazone (ros) (Alexis Biochemicals, San Diego, CA) or 10–100 ng/ml human recombinant IGF-I (Sigma-Aldrich, Milan, Italy). Ten micromoles deoxycholic acid (DCA, Sigma-Aldrich) were used as a positive control of the anti-apoptotic effect of GH (27, 28). Assessment of apoptosis by annexin V, 7-actinomycin D (7-AAD), or DNA fragmentation assay was done after a 30-min to 24-h culture period (see below). Samples of normal skin were obtained during plastic surgery and were used as a negative control.

**DNA fragmentation assay**

Quantitative determination of fragmented DNA in cytoplasm was assessed using the cell death detection ELISA plus (Roche Diagnostics, Indianapolis, IN). This assay detects the amount of histone-associated DNA fragments. The assay was performed according to the supplier’s manual; briefly, 10⁴ cells were grown in a 96-well culture plate for 24 h, starved for 12 h, and then incubated with GH, cig (or ros), or both for 30 min to 24 h as indicated in the cell culture and cell preparation section.

**Antibodies**

Human polyclonal anti-Bcl2, anti-TNF-related apoptosis-induced ligand (TRAIL), anti-BAX, anti-GH receptor (GHR) (clone SC-461), anti-IGF-I receptor (IGF-IR), anti-signal transducer and activator of transcription (STAT)5b, and anti-PPARγ antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phosphoserine antibody was purchased from Qiagen (Hilden, Germany).

**Immunoblotting**

Cells (9 × 10⁴) were washed in PBS and lysed in lysis buffer [50 mM Tris-HCl (pH 6.8), 10% glycerol, 2.5% sodium dodecyl sulfate, 10 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride]. Protein concentration was measured by Bradford assay using the Bio-Rad reagent (Bio-Rad Laboratories, Hercules, CA). For detecting phosphorylated PPARγ, nuclear extracts were immunoprecipitated with an anti-PPARγ antibody (Ab) before resolving on SDS-PAGE. Proteins (25 µg) were resolved by 12% SDS-PAGE, transferred onto nitrocellulose membrane, and stained with red ponceau to verify the amount of protein per lane. The membranes were incubated overnight at 4 °C in 50% Tris-buffered saline (TBS) [200 mM Tris-HCl (pH 7.6), 1.4 mM NaCl] and 5% TTBs (TBS, 0.05% Tween 20), containing 5% nonfat dry milk, and subsequently incubated with the primary antibody for 1 h at room temperature. After extensive washing in TTBs, a horseradish peroxidase-conjugated antirabbit IgG was added for 1 h. After four washings of the membranes in TTBs and TBS, proteins were detected using enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Piscataway, NJ).

**Transfections**

The pBLCAT₂ΔPRE vector containing the a single copy of the PPAR response element (PPRE) of the acylCoA-oxidase gene has been described (31). The p5CS-5-bPPARγ plasmid containing the human cDNA of the human PPARγ gene was kindly provided by Dr. Beatrice Desvergne (Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland). 2 × 10⁵ cells (Caco2 or NIH3T3, as specified) were transiently transfected with 10 µg pBLCAT₂ΔPRE plasmid or the empty parental vector, 3 µg p5CS-5-bPPARγ (as appropriate), and 1 µg PCH1 plasmid containing the β-galactosidase gene, using the calcium phosphate method; the latter plasmid was used to account for the variability in transfection efficiency. Cell extracts were prepared 48 h later and chloramphenicol acetyl transferase (CAT) assay and β-gal assay performed as previously described (31). Results were expressed as arbitrary units considering 100% the value obtained in absence of cig. Data represent the mean ± SD of five independent experiments, each performed in triplicate.
Real-time PCR

Real-time PCR was performed as described (32). The primer set amplifies an 83-bp fragment, the identity of which was confirmed by DNA sequencing. A control in which reverse transcription was omitted before PCR amplification was always included to eliminate the possibility that any amplification was due to contaminating genomic DNA. Experiments were carried out in triplicate; data are expressed as number of copies of PPARγ.

Statistics

Data were expressed as mean ± sd. Comparison of parameters was performed by ANOVA.

Results

GH receptor expression

GH receptor expression on Caco2 cell membranes was evaluated by radioligand binding assay. High-affinity binding (1.53 × 10⁻¹⁰ M) of ¹²⁵I-human recombinant GH was detected (Fig. 1), similar to that described for GHR in rat gastrointestinal tract (33). Preincubation of cells with a monoclonal antibody against GHR resulted in 89% inhibition of specific binding (data not shown). The number of receptors (capacity) was in the range of 4–6 fmol/mg of proteins.

Inhibition of apoptosis by GH

The level of apoptosis of starved Caco2 cells assessed by 7-AAD, histone-associated DNA fragments, or annexin V-PI, was 7.5 ± 0.9, 8.5 ± 1.1, and 10.3 ± 1.5%, respectively. The effects of GH on apoptosis of Caco2 cells was evaluated after a time range of 30 min to 24 h in serum-free medium. The fraction of apoptotic cells decreased after exposure to GH for up to 24 h by 45% (from 7.5 ± 0.9 to 3.5 ± 0.8%, as assessed by 7-AAD), 8.5 ± 1.1 to 4.8 ± 0.7% as assessed by histone-associated DNA, and 10.3 ± 1.5 to 5.2 ± 1.1% by annexin V (Fig. 2, A and B) in a dose-dependent manner (P = 0.003) (Fig. 2C); similar results were obtained using a different human colonic cancer cell line (HT29) (Fig. 2, A and C). The inhibition of apoptosis in both cell lines (Caco2 or HT29) was comparable with that obtained with 10 μM DCA for 30 min (Fig. 2A). In addition, apoptosis was evaluated 24 h after incubation with either a single dose of GH or repeated administration (every 6 h for 24 h) of GH; the latter modality of GH exposure was done in an attempt to reproduce in vitro the pulsatility of GH secretion occurring in vivo. However, each modality of GH administration was associated with a similar apoptosis rate (Fig. 2D).

GH reduces apoptosis through a IGF-I-dependent and IGF-I-independent mechanism

To assess whether the antiapoptotic effects of GH were mediated through local production of IGF-I, apoptosis in Caco2 cells was measured incubating the cells in the presence of GH alone or in combination with GHR-Ab or IGF-IR-Ab. The antiapoptotic effect of GH was reduced by blocking GH action using a specific anti-GHR-Ab (P < 0.0001); addition of IGF-IR-Ab to GH-containing cell medium reduced, albeit not abolishing, the antiapoptotic effect of GH (P < 0.03 vs. untreated cells, P < 0.006 vs. GH-treated cells), thus suggesting that IGF-I participated to the GH-dependent reduction of apoptosis. The antiapoptotic effect of IGF-I was confirmed by the reduction of apoptosis when it was added to the cell medium, and by the reversal of the effect by the simultaneous incubation with IGF-IR-Ab (Fig. 3). The fact that the level of apoptosis of cells treated with GH and IGF-IR-Ab and that of the cells treated with IGF-I is different (P < 0.05) lends further support to the notion that a quote of antiapoptotic effect is directly mediated by GH and does not require local IGF-I production (Fig. 3).

Expression of PPARγ

The expression of PPARγ in Caco2 cells was studied by Western blot. Nuclear extracts were incubated with a specific anti-PPARγ antibody; extracts from normal colonic mucosa and in vitro synthesized PPARγ were used as positive controls, those from NIH3T3 cells and samples of normal human skin as negative controls (Fig. 4A). To evaluate the functional integrity of the receptor, a reporter plasmid containing a PPAR response element fused to a heterologous promotor and a reporter gene was transiently transfected into Caco2 cells. Addition of 1 μM cig increased the basal level of expression of a reporter plasmid containing a single copy of a PPRE by 2-fold; exposure to higher cig concentrations (10 μM) was not associated with increased levels of CAT (Fig. 4B). Exposure of NIH3T3 cells, which do not express detectable levels of PPARγ, to cig did not enhance the CAT activity of the reporter plasmid unless cells were cotransfected with a PPARγ-containing expression plasmid (Fig. 4B), thus suggesting the specificity of action of PPARγ activator.
Antagonistic Effects of PPARγ and GH

A

Untreated CaCo-2 cells
Apoptosis 10.3%

GH-treated CaCo-2 cells
Apoptosis 5.2%

DCA-treated CaCo-2 cells
Apoptosis 5.0%

Untreated HT29 cells
Apoptosis 19.0%

GH-treated HT29 cells
Apoptosis 7.6%

DCA-treated HT29 cells
Apoptosis 9.0%

B

Untreated CaCo-2 cells
Apoptosis 7.5%

GH-treated CaCo-2 cells
Apoptosis 3.5%

C

% Inhibition of apoptosis

0  0.05  0.5  5  50
GH (nM)

D

% Inhibition of apoptosis

30 min  1 h  6 h  24 h  24 h

Single dose  Repeated doses
Enhanced apoptosis by PPARγ activation

Caco2 cells exposed to 0.1–100 μM of either cig or ros for up to 24 h exhibited an increased apoptosis rate (Fig. 5, A and B). Apoptosis increased from 8 ± 0.5 to 15 ± 0.4% just after 30 min, as assessed by annexin V PI method (Fig. 5A), and in a dose-dependent manner up to 50 μM thiazolidinediones (TZDs) as assessed by measuring the amount of histone-associated DNA fragments (P < 0.001) (Fig. 5B). Because exposure of Caco2 cells to 100 μM TZDs increased necrosis but not apoptosis (Fig. 5A), the next experiments were performed using 50 μM TZDs (Fig. 5, A and B). The effect of cig on apoptosis was not due to the drug toxicity because it was ineffective in NIH3T3 cells not expressing PPARγ (Fig. 5B). Exposure to up 50 μM cig increased apoptosis leaving un-affected necrosis. Thus, 50 μM of either cig or ros was used in subsequent experiments (Fig. 5, A and B). Caco2 cells cultured in the presence of 0.5–50 nM GH exhibited an increased apoptosis up to 3.5-fold when exposed to 50 μM cig (P < 0.03) (Fig. 5C); at variance, cig increased apoptosis of GH-treated cells by only 80% at 24 h; this apparent discrepancy could be explained, taking into account that GH reduced the phosphorylation of PPARγ, which, in turn, might enhance its activity; this effect was present at 30 min and no longer detectable after 24 h (Fig. 5D); thus, cig was capable of overcoming the antiprototic effect of GH in Caco2 cells.

Effects of GH on PPARγ expression

Serum-deprived Caco2 cells treated with 50 nM GH for 6 or 24 h had a reduced number of PPARγ transcripts as assessed by either real-time PCR (Fig. 6A) or Western blot (Fig. 6B). After 6 and 24 h incubation with GH, the transcripts of PPARγ decreased from 5126 ± 798 to 914 ± 411 (P < 0.002) and from 14,691 ± 611 to 6767 ± 2090 (P < 0.05), respectively (Fig. 6A); the GH-dependent down-expression of PPARγ was confirmed in HT29 cells by either RT-PCR (data not shown) or Western blot (Fig. 6C). The mechanisms underlying the negative effects of GH on PPARγ expression was further investigated evaluating the expression of STAT5b

FIG. 2. Inhibition of apoptosis by GH treatment. Cells were starved for 12 h and then exposed to 0.05–50 nM GH for up to 24 h. The rate of apoptosis was evaluated by annexin V PI (A) or 7-AAD methods (B) or by measuring the amount of fragmented DNA by the cell death kit as reported in Materials and Methods (C and D). A, Apoptotic cells, enclosed in the R2 box, decreased from 10.3 to 5.2% (19.0 to 7.6% in HT29 cells) after GH exposure as assessed by annexin V PI assay, similar to the antiprototic agent DCA (10 μM). B, Reduction of apoptosis from 7.5 to 3.5% in GH-treated Caco2 cells evaluated by the 7-AAD method (apoptotic cells are enclosed in the R2 box). C and D, The fraction of apoptotic cells (either Caco 2 [empty columns] or HT29 cells [filled columns]) decreased after exposure to 0.05–50 nM GH for either 30 min (C) or 24 h (D) by 30–40% (P = 0.003). Results are expressed as mean ± SD of five different experiments, each performed in triplicate.
FIG. 5. Activation of PPARγ increases Caco2 apoptosis and reverts GH-dependent increase of Caco2 survival. Cig or ros was added to the culture medium at 0.1–100 μM alone (A and B); because 50 μM cig determined the maximal increase in apoptosis leaving unaffected necrosis, subsequent experiments were performed with this dose of cig. Treatment with up to 50 μM cig significantly increased apoptosis of Caco2 cells from 8 ± 0.5 to 14 ± 0.4% but not necrosis, just after 30 min, as assessed by annexin V PI method; stained cells positive for annexin V and negative for PI were considered apoptotic. The samples were measured by FITC/PI flow cytometry on a FACS apparatus (A); cig or ros increased apoptosis up to 170% in untreated Caco2 cells in a dose-dependent manner (P < 0.03) (B) and in GH-treated cells up to 3.5-fold (P < 0.001) (C), as assessed by measuring the amount of histone-associated DNA fragments (see Materials and Methods). This paradoxical effect of GH was observed only after a short exposure of the cells to GH and probably was due to a reduction of phosphorylation of PPARγ(D); nuclear extracts were precipitated with an anti-PPARγ antibody, resolved on a 12% SDS-PAGE, blotted onto nitrocellulose membrane, and Western blot performed with an anti-phosphoserine antibody.
protein; HT29 cells exposed to 5–50 nM GH for 6–24 h enhanced the expression of STAT5b (Fig. 7).

**Effects of GH or PPARγ activator on apoptosis-associated proteins**

Caco2 cells were treated with 0.5–50 nM GH or 1–50 μM cig for 6–24 h. Cellular extracts were immunoblotted with antibody against Bcl-2, TRAIL, and Bax. Expression of Bcl-2 and TRAIL was not affected by either 50 nM GH or 50 μM cig treatment after 24 h; at variance, GH reduced the level of expression of Bax, which was increased by treatment with cig (Fig. 8A). The lower level of expression of Bax protein was revealed in 0.5–50 ng/ml GH-treated cells after 6 and 24 h.

**Discussion**

Patients with acromegaly have an increased prevalence of colonic polyps (34, 35), and a link between colonic polyps and activity of the disease has been postulated on clinical grounds (36). Exposure of several cell lines to GH has been associated with reduced apoptosis (1–6) and possibly with progression of neoplasms (3). GHR is widely expressed in several organs, including the gastrointestinal tract (30, 37, 38). Our study confirms that in Caco2 colonic-derived cells, high-affinity GH binding sites are present on the cytoplasmic membranes; albeit being expressed at a low density, GHRs were sufficient to drive GH action. GH actions depend on its binding to a membrane receptor that belongs to the cytokine receptor superfamily (38, 39). It was postulated that GH might cause prolonged survival of intestinal epithelial cells, based on studies of transgenic mice overexpressing bovine GH, in which the observed increased mucosal mass was not associated with an enhanced crypt cell production rate and DNA-specific activity (10); GH might also protect intestinal cells from apoptosis induced by radiation (40). GH seems to reduce apoptosis in many cell lines (3, 6, 15), but a direct involvement in colonic cells is largely speculative.

Using the pro-B murine Ba/F3 cell line expressing GH receptor, Baixeras et al. (41) reported that GH-dependent proliferation and survival of these cells were not mediated by IGF-I. The GH-dependent antiapoptotic effects were mediated by the NF-κB transcription factor. Our results demonstrated that the antiapoptotic effects of GH in Caco2 cells is not completely dependent on local production of IGF-I, albeit part of GH action might be counteracted by an anti-IGF-IR antibody. The molecular mechanisms underlying the anti-
apoptotic effects of GH might include a sustained production of Bcl-2 and NF-κB, as shown in Ba/F3 cells (8), or an increased homeo box A1-dependent expression of Bcl-2 as reported in human mammary carcinoma cells (1). At variance, in blastocysts GH treatment did not affect Bcl-2 expression but reduced the expression of the proapoptotic Bax proteins (5). In addition, GH treatment inhibited apoptosis in human leukemic cells and Chinese hamster ovary cells through activation of Akt phosphorylation, leaving unchanged the Bcl-2 levels (6). We herein reported that GH-dependent prolonged survival of Caco2 cells is mediated by a marked reduction of the proapoptotic Bax protein expression, without effect on Bcl-2; thus, the antiapoptotic effects of GH in this cell line may derive from a change in the Bax/Bcl-2 ratio. However, effects of GH on the regulation of pathways involving other proteins of the apoptotic process cannot be ruled out.

PPARγ is considered a tumor suppressor gene, the activation of which leads to cellular differentiation and apoptosis (14, 16–20, 23–25). Loss of heterozygosity and somatic mutations in the PPARγ gene might be an early event in colonic tumorigenesis (42–44); our recent findings of a reduced PPARγ expression in the colonic mucosa of patients with active acromegaly or colonic polyps lend support to this notion (32, 45). Thus, the lower levels of expression of PPARγ in Caco2 cells treated with GH might contribute to the antiapoptotic effects of GH in this cell line. Serum deprivation is associated with changes in the expression of several transcription factors, including PPARα (46). Our findings suggest that a link between starvation and increased PPARγ levels might exist. However, the underlying mechanisms remain unknown. Furthermore, the different expression of PPARγ did not affect the GH-dependent down-regulation of this nuclear receptor. However, exposure of Caco2 cells, either GH treated or untreated, to cig increased apoptosis by about 3-fold; this suggests that even though reduced by GH treatment, PPARγ activation is able to overcome the antiapoptotic effects of GH. GH treatment was associated with a paradoxical increase of PPARγ-dependent apoptosis during the first half-hour; this might be due to the GH-dependent reduction of PPARγ phosphorylation, as shown by Western blot, which was no longer present after 24 h. In the colonic cancer HT-29 cell line, activation of PPARγ by troglitazone increased cell death associated with down-regulation of c-myc and up-regulation of c-jun and growth arrest and DNA damage-inducible gene 153 (31); at variance, in papillary thyroid cancer activation of PPARγ led to increased c-myc expression (30).

The levels of expression of Bcl-2, Bax, and p53 have been shown to be increased, unchanged, or decreased (47–49), likely depending on the cell lines. Activation of PPARγ in Caco2 cells increased the expression of Bax, leaving unaffected that of Bcl-2 and TRAIL, thus suggesting that, at least in this cell line, GH and PPARγ regulate apoptosis through a common pathway; alternatively, the reduced levels of expression of Bax found in GH-treated cells might be due to a down-regulation of PPARγ by GH; GH treatment was associated with a reduced level of PPARγ and an increased expression of STAT5b; albeit a direct link could not be proven from our studies, it is likely that STAT5b might be responsible for the GH-dependent down-regulation of PPARγ in colonic cells. Recent papers (50, 51) have demonstrated that the cross-talk between PPAR and STAT pathways is bidirectional, suggesting a mechanism whereby PPAR ligands or

**Fig. 8.** Activation of PPARγ is associated with changes in the expression of apoptotic factors. Caco2 cell extracts were prepared as described in Materials and Methods. A, Cells were treated with 50 nM GH or 100 μM cig for 24 h. Western blot was done using anti-Bcl-2-, anti-TRAIL-, and anti-Bax-specific antibody. Activation of PPARγ was associated with increased levels of expression of Bax, leaving unaffected Bel-2 and TRAIL expression. GH did not modify the expression of TRAIL and Bel-2 but reduced the expression of Bax. The down-regulation (B) and up-regulation (C) of Bax in cells treated with GH and cig, respectively, was confirmed by dose- and time-course experiments.
GH might interact to each other. Shipley et al. (51) reported that GH treatment of COS-1 cells transfected with PPARγ, STA5b and GHR was not associated with changes in the level of expression of STA5b protein evaluated after 4 h. At variance with the report of Shipley and Waxman, our results show that GH increased STA5b levels in Caco2 cells after 6 and 24 h. This discrepant result may be due to the different cell line, the exposure time to GH, and the fact that we did not transfect the cells with a STA5b-containing plasmid.

In conclusion, we have reported a direct involvement of GH in prolonging survival of colonic cells, which might be opposed by activation of PPARγ. These data provide a molecular basis to the effects of GH on colonic epithelial cells and suggest a potential role of PPARγ agonists as chemopreventive agents in acromegalic patients at high risk of developing colonic neoplasms.

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Address all correspondence and requests for reprints to: Fausto Bogazzi, Dipartimento di Endocrinologia e Metabolismo, Università di Pisa, Ospedale di Cisanello, Via Paradisa 2, 56124 Pisa, Italy. E-mail: f.bogazzi@end.med.unipi.it or f.bogazzi@hotmail.com.

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