Interleukin-1 β (IL-1 β) and IL-6 modulate insulin-like growth factor-binding protein (IGFBP) secretion in colon cancer epithelial (Caco-2) cells

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Abstract

Chronic inflammation is characterised by modifications in cytokine concentrations, whereas growth is mainly dependent on the GH-IGF axis. IGF-I bioavailability is modulated by a family of IGF-binding proteins (IGFBPs). The aim of the present study was to evaluate the interactions among interleukin-1 β (IL-1 β), IL-6 and IGFBP secretion by intestinal cells to assess whether cytokines modulate IGFBP secretion, and in turn IGF-I and IGF-II bioavailability. The human colon carcinoma derived cell line Caco-2 was used as an *in vitro* model for its capacity to differentiate spontaneously. Experiments were carried out on day 4 (undifferentiated state) and day 14 (differentiated state) after plating. Carcinoembryonic antigen (CEA) was used as a marker of differentiation and increased in the conditioned media (CM) from days 4 to 14 (0.2 ± 0.01 ng/ml per 10^5 cells vs 3.3 ± 0.2 ng/ml per 10^5 cells, $P < 0.05$). IGFBP-2 and IGFBP-4 secretion decreased concomitantly. Cells were stimulated with IL-1 β and IL-6 at 1, 10 and 50 ng/ml, and with IL-1 β and IL-6 in combination at the same dose of 1 and 10 ng/ml. IGF-I at 50 ng/ml was used as a control. Caco-2 cells expressed and secreted mainly IGFBP-2 and IGFBP-4 into the CM. On day 4, IL-1 β (1 ng/ml) and IL-6 (10 and 50 ng/ml) reduced IGFBP-2 by $29 \pm 8\%$, and by 32 ± 9 and $38 \pm 8\%$ respectively ($P < 0.05$). IGFBP-4 was also

reduced by IL-1 β at 1 and 50 ng/ml ($-14 \pm 4\%$ and $-46 \pm 11\%$ vs serum free medium (SFM) respectively, $P < 0.05$), and IL-6 at 50 ng/ml ($-46 \pm 15\%$, $P < 0.05$). Both IGFBP-2 and IGFBP-4 were reduced by IL-1 β and IL-6 in combination at 1 and 10 ng/ml ($P < 0.05$). On day 14, IGFBP-2 band intensity was reduced at 10 ng/ml of IL-1 β ($-22 \pm 15\%$ vs SFM, $P < 0.05$) and at 50 ng/ml of both cytokines ($-33\% \pm 8\%$ and $-13\% \pm 13\%$ vs baseline respectively, $P < 0.05$). IGFBP-4 band intensity decreased with 10 and 50 ng/ml of IL-1 β ($-35 \pm 11\%$ and $-46 \pm 15\%$ vs SFM respectively) and IL-6 ($-36\% \pm 10\%$ and $-46 \pm 15\%$ vs SFM respectively). IL-1 β and IL-6 in combination at 1 and 10 ng/ml reduced both IGFBP-2 and IGFBP-4.

In conclusion, IGFBP-2 and IGFBP-4 secretion in CM decreased with Caco-2 cell differentiation. IGFBP-2 and IGFBP-4 were significantly decreased by IL-1 β and IL-6 treatment in both the undifferentiated and differentiated state. Furthermore, these cytokines increased cell proliferation whereas total protein content was significantly reduced only at the higher concentrations of IL-6 and IL-1 β . These findings suggest that interleukins modulate the IGF-IGFBP system in Caco-2 cells *in vitro*.

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Introduction

Delayed linear growth and pubertal development are frequent in children with chronic inflammatory bowel diseases (CIBD) (Motil *et al.* 1993, Savage *et al.* 1999). The growth impairment can be ascribed to various factors such as malnutrition (Kelts *et al.* 1979), prolonged treatment with steroids (Allen 1996), and chronic inflammation. Growth is largely dependent on the growth

hormone–insulin-like growth factor (GH-IGF) axis, and IGF-I and IGF-II are fundamental for cell proliferation and differentiation. The biological activities of these peptides are modulated mainly by the insulin-like growth factor-binding proteins (IGFBPs). The effects of chronic inflammation, and in particular of its mediators, on these proteins have not been fully investigated.

Proinflammatory cytokines and IGFBP interactions have been shown in previous studies (Yateman *et al.* 1993,

Katz *et al.* 1995, Olney *et al.* 1995, Wang *et al.* 1995, Gentilini *et al.* 1998). These studies showed interactions that were cell specific, and studies in adult rats have shown that the effects of cytokines on IGFBPs could be also organ specific (Wood *et al.* 1995).

CIBDs are characterised by increased interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) concentrations both in the intestinal mucosa and in the serum of affected subjects (Mahida *et al.* 1989, Brynskov *et al.* 1992, Pullman *et al.* 1992, Stevens *et al.* 1992, Reimund *et al.* 1996). Furthermore, increased serum IL-6 has been associated with low IGF-I levels and growth delay in the rat (De Benedetti *et al.* 1997).

Modifications in mucosal IGFBPs could account for the intestinal mucosal damage that occurs in CIBD, and altered IGF-I bioavailability for the impaired growth frequently observed in these patients. Thus, the aim of the present study was to evaluate the interactions among IL-1 β , IL-6 and IGFBP secretion by intestinal cells to assess whether cytokines may modulate IGFBP secretion and in turn IGF-I and IGF-II bioavailability.

The human colon carcinoma derived cell line Caco-2 was used as the *in vitro* model for its capacity to differentiate spontaneously *in vitro* under established conditions (Pinto *et al.* 1983, Oguchi *et al.* 1994). This cell line provides an appropriate model to investigate the effects of cytokines on the IGF system in the gut as these cells are also known to express and secrete IGF-I, IGF-II and IGFBPs throughout proliferation and differentiation (Oguchi *et al.* 1994, Singh *et al.* 1994, 1996). In addition, these cells have been shown to express the IGF-I receptor and cytokine receptors on their cell surface (Molmenti *et al.* 1993, Hoefflich *et al.* 1996a, Parikh *et al.* 1997).

Materials and Methods

Cell culture

The Caco-2 cells were obtained from the ECACC (European Collection of Cell Cultures, Salisbury, Wiltshire, UK). Cells were used between passages 20 and 55. Cells were grown in Dulbecco's Modified Eagle's medium (DMEM, Sigma, Aurora, OH, USA) supplemented with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100 000 U/l penicillin, 100 mg/l streptomycin and 10% heat-inactivated fetal calf serum under humidified atmosphere at 37 °C, 5% CO₂. The conditioned media (CM) was collected at 4, 7, 9, 11, 14, 17, 19 and 21 days after plating. Before collection, the cells were washed with serum free medium with 0.1% bovine serum albumin (SFM), and incubated in SFM for 24 h. The effects of cytokines on IGFBP secretion were carried out on day 4 (undifferentiated state) and day 14 (differentiated state) after plating. Cells were plated into 24-well plates at a density of 2×10^4 cells/cm². Twenty-four hours prior to stimulation, the monolayers were washed

and incubated in SFM. After this incubation period, cell monolayers were stimulated with IL-1 β and IL-6 (PeproTech EC Ltd, London, UK) at 1, 10 and 50 ng/ml. These interleukins were added in combination at 1 and 10 ng/ml in SFM with 0.1% BSA. IGF-I (kindly provided by Pharmacia, Stockholm, Sweden) was used as a control at 50 ng/ml. The CM were collected after a 48-h incubation, concentrated 20-fold by centrifugation using Ultrafree-MC Centrifugal Filter Units (Millipore M-0411, Sigma, St Louis, MO, USA), and kept at -20 °C until further assayed. Experiments were carried out in triplicate and the cell number was determined at the end of each experiment.

Determination of the carcinoembryonic antigen

Carcinoembryonic antigen (CEA) was used as a marker of cell differentiation, and was assayed in the CM from day 4 and day 14 by an IRMA assay (DiaSorin, Turin, Italy). The intra-assay coefficient of variation was 2.9%, and the inter-assay coefficient of variation 7.3%. The concentration in ng/ml was adjusted for 10⁵/cells.

Western ligand blotting

Equal aliquots of the CM concentrated 20-fold were subjected to electrophoresis on 12.5% acrylamide gels (Laemmli 1970). The proteins were then transferred to nitrocellulose membranes and probed with ¹²⁵I-IGF-I (Amersham Pharmacia Biotech, Amersham, Bucks, UK) as described previously (Camacho-Hübner *et al.* 1992). The IGFBPs were identified by immunoblotting, probing the nitrocellulose membranes with specific antibodies against IGFBP-2 (Upstate Biotechnology, New York, NY, USA; 1:2000 dilution), IGFBP-4 (Upstate Biotechnology; 1:500 dilution) and IGFBP-6 (GroPep Ltd, Adelaide, Australia; 1:1000 dilution) at 4 °C overnight. The IGFBP-6 immunoblotting was performed to identify a third IGFBP that showed in some blots. The bands were visualised by enhanced chemiluminescence (ECL) as recommended by the manufacturer (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK).

The changes in IGFBP band intensity were evaluated on the Western ligand blots, as the IGFBP-4 antibody showed some cross-reactivity with IGFBP-2, using the Imaging Densitometer BIORAD GS-670 (Bio-Rad Laboratories, Hercules, CA, USA), and expressed in optic densitometry units (ODU) adjusted per 10⁵ cells. The data were then analysed as percentage change with respect to the basal value. Normal human serum was used as the standard reference value.

Protein assay

The assay was performed both in the undifferentiated and differentiated state. Cells were washed, starved for 24 h

and then incubated with the stimuli for 48 h. IGF-I (50 ng/ml) was used as a control and the cytokines were used at 1 and 10 ng/ml. At the end of the experiment the cells were counted and the proteins were subsequently extracted using modified RIPA buffer (Tris-HCl: 50 mM, pH 7.4, NP-40: 1%, Na-deoxycolate 0.25%, NaCl 150 mM, EDTA 1 mM, PMSF 1 mM, aprotinin, leupeptin and pepstatin 1 µg, Na₃VO₄ 1 mM, NaF 1 mM). The lysate was stored at -20 °C until assayed. The total protein content was determined using the 'microassay Bio-Rad' protocol (Bio-Rad Lab, Munich, Germany). Briefly, a standard curve was prepared using bovine albumin (Bio-Rad Lab, Hercules, CA, USA). To determine the concentration, the dye was added to each sample and standard and the absorbance was read at 570 nm. The total protein content was expressed in µg/ml and adjusted for cell number.

DNA synthesis

DNA synthesis was used as an index of cell proliferation and was assessed by bromodeoxyuridine (BrdU) incorporation in the absence and presence of the stimuli. For these experiments cytokines were used at 1 and 10 ng/ml individually or in combination. IGF-I was used as positive control and SFM as negative control. Background and blanks were taken into account. Cells were plated at 10⁵ cells/well (96-well plates). Plating efficiency was determined at cell attachment and was always greater than 80%. At the same time cells were washed and incubated with SFM 12 h prior to stimulation. Proliferation was assessed after 12, 24, 36 and 48 h of incubation. Maximum proliferation was detectable after 48 h of incubation, thus only these results are reported.

BrdU incorporation was determined using a commercial kit (Oncogene, Cambridge, MA USA). The absorbance was assessed using a dual wavelength (450–540 nm) and expressed as ODU.

Isolation of RNA

In order to study gene expression of IGFs and IGFBPs, total RNA was extracted from confluent cells using the TRI reagent (Sigma Aldrich Company Ltd, Poole, UK) according to the procedure of Chomczynski (Chomczynski 1993). The cells were plated at 2×10^4 cells/cm² into six-well plates. Extractions were performed on days 4 and 14 after a 48-h incubation in SFM. RNA integrity was verified by fractionating on 1% MOPS-agarose gels, transferred onto nylon membranes and then stained with methylene blue.

RT-PCR

The baseline expression of genes for IGFBP-2, IGFBP-3 and IGFBP-4 were studied from days 4 to 15 to include

both stages of differentiation. IGF-I and IGF-II gene expression was studied until day 23 after plating. Aliquots of 5 µg of total RNA were amplified and transcribed to cDNAs by random priming using the Superscript RNase H⁻ reverse transcriptase (Gibco BRL Life Technologies, Carlsbad, CA, USA) to prime a standard reverse transcriptase (RT) reaction (Feinberg & Vogelstein 1984). The cDNAs of interest were selectively amplified by the polymerase chain reaction using the DNA polymerase DyNAzyme II (Finnzymes, Espoo, Finland) using primers based on published sequences (Shimasaki & Ling 1991).

Statistics

The statistical analysis was performed using SPSS 7.5 for windows. Data are expressed as means ± S.E.M. unless stated otherwise. The normal distribution of the data was determined using the Kolmogorov-Smirnov test. Data were analysed by ANOVA followed by Scheffé's test. $P < 0.05$ was considered significant.

Results

Characterisation of Caco-2 cells

The 34 kDa protein IGFBP-2 and the 24 kDa protein IGFBP-4 were first identified by Western ligand blotting and subsequently by immunoblotting in 48 h CM. A third band of 28–34 kDa was identified as IGFBP-6 and was not analysed further in this study.

IGFBP-2 secretion decreased from days 4 to 14 after plating (10.42 ± 4.8 vs 4.76 ± 0.4 relative ODU, NS), and increased thereafter but not significantly. IGFBP-4 showed a similar pattern with a more pronounced decrease as proliferation and differentiation increased (40.9 ± 0.5 on day 4 vs 8.38 ± 0.2 on day 14, $P < 0.05$) (Fig. 1A). The lowest concentrations were achieved on day 17 after plating (5.7 ± 0.4).

The levels of the phenotypic marker of cell differentiation CEA, adjusted for cell number, rose significantly in the CM from day 4, peaking at day 14 (0.2 ± 0.01 ng/ml per 10⁵ cells vs 3.3 ± 0.2 ng/ml per 10⁵ cells, $P < 0.05$) (Fig. 1B).

Cell number increased significantly during time-course experiments from days 4 to 14 ($3.2 \pm 1.33 \times 10^5$ vs $20.66 \pm 1.9 \times 10^5$ /cells, $P < 0.05$) and decreased thereafter (Fig. 1C).

In consideration of IGFBP-2 levels in CM, CEA concentration, and of cell proliferation over time, experiments were carried out on days 4 and 14. The genes for IGF-I, IGF-II, IGFBP-2 and IGFBP-4 were expressed from days 4 to 15. IGFBP-3 gene expression and secretion were not detected at any given time point (Fig. 2).

Figure 1 (A) IGFBP-2 (dotted line) and IGFBP-4 (solid line) mean concentrations in CM at different time-points after plating. The data are the means of three experiments in triplicate wells and are expressed as relative ODU (adjusted for 10⁵ cells). * $P < 0.05$ vs day 14. (B) CEA concentration in the CM from Caco-2, adjusted for cell number (ng/ml per 10⁵ cells) at different time-points after plating. The concentration was highest on day 14, and significantly different from baseline. * $P < 0.05$ vs day 4. The data represent the means $\pm$ S.E.M. of four experiments in triplicate wells. The solid line represents the mean and the dotted lines $\pm$ S.E.M. (C) Variations in cell number of Caco-2 cells during the time-course experiments. After day 14, cell number decreased owing to spontaneous cell death. * $P < 0.05$ vs baseline. The data represent the means $\pm$ S.E.M. of four experiments in quadruplicate wells. The solid line represents the mean and the dotted lines $\pm$ S.E.M.

Figure 2 Agarose gel electrophoresis of PCR products of Caco-2 cells using primers for IGF-I, IGF-II, IGFBP-2, IGFBP-3 and IGFBP-4 genes. Molecular sizes of products were established in base pairs. Samples were prepared as described in Materials and Methods and total RNA obtained at the indicated times. RNA extracted from fibroblasts characterised in our laboratory was used as a positive control. IGFBP-2, -3 and -4 genes were studied until day 15, IGF-I and IGF-II genes until day 23 after plating.

Effects of IL-1 β and IL-6 on IGFBP secretion by Caco-2 cells in the undifferentiated state (day 4)

IGFBP-2 levels were significantly reduced at 1 ng/ml of IL-1 β by $29 \pm 8\%$ ($P < 0.05$) (Fig. 3A) and at 10 and 50 ng/ml of IL-6 by $32 \pm 9\%$ and $38 \pm 8\%$ ($P < 0.05$) (Fig. 3C).

IGFBP-4 was also reduced by IL-1 β at both low and high concentrations in a dose-dependent fashion (1 and 50 ng/ml; $14 \pm 4\%$ and $46 \pm 11\%$ vs SFM respectively, $P < 0.05$, Fig. 3B), and by IL-6 at 50 ng/ml ($-46 \pm 15\%$, $P < 0.05$, Fig. 3D).

When IL-1 β and IL-6 were used in combination at the same dose, IGFBP-2 was significantly reduced at 1 and 10 ng/ml ($-34 \pm 3\%$ and $-62 \pm 15\%$ vs baseline respectively, $P < 0.05$). IGFBP-4 was significantly reduced after stimulation with IL-1 β and IL-6 in combination at the same dose of 1 and 10 ng/ml (-41 ± 12 and $-59 \pm 12\%$ vs baseline respectively, $P < 0.05$) (Fig. 3).

IGF-I at 50 ng/ml was used as internal control and reduced significantly both IGFBP-2 and IGFBP-4 band intensities (Fig. 3).

Effects of IL-1 β and IL-6 on IGFBP secretion by Caco-2 cells in the differentiated state (day 14)

Caco-2 cells were also studied in the differentiated state. IGFBP-2 band intensity was reduced by IL-1 β at 10 and 50 ng/ml ($-22 \pm 15\%$ and $33 \pm 8\%$ respectively, $P < 0.05$) and by IL-6 at 50 ng/ml ($-13 \pm 13\%$; $P < 0.05$) (Fig. 4A,C).

In contrast, IGFBP-4 was significantly reduced by both interleukins at 10 and 50 ng/ml. IL-1 β reduced IGFBP-4

by $35 \pm 11\%$ at 10 ng/ml, and by $46 \pm 15\%$ at 50 ng/ml ($P < 0.05$; Fig. 4B). Similarly, IL-6 reduced IGFBP-4 by $36 \pm 10\%$ at 10 ng/ml, and by $46 \pm 15\%$ at 50 ng/ml ($P < 0.05$; Fig. 4D). IGF-I at 50 ng/ml reduced significantly both IGFBP-2 and IGFBP-4 (Fig. 4).

IGFBP-2 was significantly reduced by IL-1 β and IL-6 in combination at the same dose of 1 and 10 ng/ml (-28 ± 7 and $-60 \pm 20\%$ respectively, $P < 0.05$). IGFBP-4 was also found to be reduced by the same concentration but statistical significance was not attained owing to the large variability among experiments (Fig. 4).

Total protein changes at both states of differentiation

Treatments reduced significantly total protein content after 48 h of incubation on day 4 at 10 ng/ml of both cytokines and if IL-1 β and IL-6 were used in combination at the same dose of 1 and 10 ng/ml. On day 14, a decrease in total protein content was observed after 48 h incubation but statistical significance was attained only if IL-1 β and IL-6 were used in combination (Fig. 5).

IGF-I determined an increase in total protein content at variance with the effect observed on IGFBP-2 and IGFBP-4 (Fig. 5).

DNA synthesis

DNA synthesis was studied in undifferentiated cells, in baseline conditions. An increase was observed in all experiments after a 48-h incubation with respect to the control (SFM). This increase was statistically significant using IL-1 β at 10 ng/ml and IL-6 at both 1 and 10 ng/ml

Figure 3 Effect of IL-1 β and IL-6 on IGFBP secretion in Caco-2 cells in the undifferentiated state (day 4). Changes in IGFBP-2 (A, C) and IGFBP-4 (B, D) secretion were determined by Western ligand blotting as described in Materials and Methods. Data are expressed as percentage of serum free band intensity (SFM) (100% minus reduction in band intensity) after stimulation with IL-1 β and IL-6 at 1, 10 and 50 ng/ml. IL-1 β and IL-6 were also used in combination at the same dose of 1 and 10 ng/ml. IGF-I at 50 ng/ml was used as a control. The data represent the means $\pm$ S.E.M. of six experiments in triplicate wells. * $P < 0.05$ vs SFM; ** $P < 0.05$ vs IL-1 β 1 ng/ml. Representative ligand blots of experiments with IL-1 β (left) and IL-6 (right) are shown in the middle. The molecular weights of radiolabelled bands estimated relative to the mobility of the standards (Amersham Pharmacia Biotech) are shown on the left-hand side and correspond to IGFBP-2 and IGFBP-4 as indicated on the right-hand side of the Figure. NHS: normal human serum.

Figure 4 Effect of IL-1 β and IL-6 on IGFBP secretion in Caco-2 cells in the differentiated state (day 14). Changes in IGFBP-2 (A, C) and IGFBP-4 (B, D) secretion were determined by Western ligand blotting as described in Materials and Methods. Data are expressed as percentage change compared with control (SFM) (100% minus reduction in band intensity) after stimulation with IL-1 β at 1, 10 and 50 ng/ml. IL-1 β and IL-6 were also used in combination at the same dose of 1 and 10 ng/ml. IGF-I at 50 ng/ml was used as a control. The data represent the means $\pm$ S.E.M. of five experiments in triplicate wells. * $P < 0.05$ vs SFM. ** $P < 0.05$ vs IL-11. Representative ligand blots of experiments with IL-1 β (left) and IL-6 (right) are shown in the middle. The molecular weights of radiolabelled bands estimated relative to the mobility of the standards (Amersham Pharmacia Biotech) are shown on the left-hand side and correspond to IGFBP-2 and IGFBP-4 as indicated on the right-hand side of the Figure. NHS: normal human serum.

Figure 5 Total protein content, on day 4 (A), was reduced significantly compared with baseline, after incubation with IL-1 β and IL-6 at 10 ng/ml and both cytokines in combination. On day 14 (B), a reduction was observed if the cytokines were used in combination at the same dose of 1 and 10 ng/ml. IGF-I at 50 ng/ml was used as a positive control, and SFM as a negative control. Total protein content was adjusted for cell number and expressed as $\mu\text{g/ml}$ per 10^4 cells on day 4 and 10^5 cells on day 14. Data are means $\pm$ S.E.M. and are the results of four experiments in triplicate wells. * P 0.05 vs SFM.

Figure 6 DNA synthesis, measured by BrdU incorporation after a 48-h incubation with IL-1 β and IL-6 at 1 and 10 ng/ml and in combination at the same doses. Data are means $\pm$ S.E.M., are expressed in ODU, and are the results of four experiments in triplicate wells. All stimuli increased proliferation significantly (* P < 0.05) compared with baseline (SFM) except for IL-1 β used at 1 ng/ml.

and did not increase further when cytokines were used in combination at the same dose of 1 and 10 ng/ml (SFM: 1.9 ± 0.1 ODU vs IL-1 β 10 ng/ml: 2.1 ± 0.6 ODU and IL-6 1 ng/ml: 2.0 ± 0.1 ODU, P < 0.05, Fig. 6). IGF-I increased significantly DNA synthesis, as expected.

Discussion

Caco-2 cells were shown to secrete mainly two IGFBPs, IGFBP-2 and IGFBP-4. Both proteins decreased in the CM, concomitantly with the increase in proliferation rate and concentration of CEA, used as a marker of differentiation. IL-1 β and IL-6 reduced both IGFBP-2 and IGFBP-4 band intensities in the CM, compared with baseline conditions and were shown to increase DNA synthesis. Total protein content was reduced by both cytokines at high concentrations (10 ng/ml) and when used in combination.

The Caco-2 cells used in the present study expressed IGFBP-2 and IGFBP-4 as previous authors have shown and identified to be the major IGFBPs secreted by epithelial intestinal cell lines (Singh *et al.* 1994). They did not express IGFBP-3 mRNA, suggesting that they possibly are a subtype from the parental line. This finding is in contrast with previous studies (Oguchi *et al.* 1994, Zhang *et al.* 1995, Hoefflich *et al.* 1996b, Park *et al.* 1996, Jehle *et al.* 1999). However, Singh *et al.* (1996) had previously shown that a subset of these cells only secreted traces of IGFBP-3.

Data in the literature concerning IGFBP-2 and -4 modifications during cell proliferation and differentiation are discordant (Oguchi *et al.* 1994, Singh *et al.* 1994, Zhang *et al.* 1995, Park *et al.* 1996, Jehle *et al.* 1999). The slight decrease in IGFBP-2 as cells proliferate and differentiate is in accordance with previous studies by Hoefflich *et al.* (1996b) and at variance with others (Oguchi *et al.* 1994, Singh *et al.* 1994, Park *et al.* 1996). The IGFBP-4 findings are similar to those of most authors (Oguchi *et al.* 1994, Zhang *et al.* 1995).

The effect of interleukins on total protein content might reflect a general regulation of proteins necessary for cell proliferation and differentiation; however, a significant reduction was found only at high doses of IL-6 and IL-1 β , and in the differentiated state this was observed only if the cytokines were used in combination whereas the effects on IGFBP-2 and -4 were seen even at low doses and both in the undifferentiated and differentiated state.

It has been shown previously that changes in IGFBP-2 and IGFBP-4 drive proliferation and differentiation in Caco-2 cells (Oguchi *et al.* 1994, Singh *et al.* 1994, Zhang *et al.* 1995, Hoefflich *et al.* 1996b, Park *et al.* 1996, Jehle *et al.* 1999). Moreover, IL-1 β and IL-6 increased DNA synthesis in Caco-2 cells, and our recent data show that IL-1 β inhibits apoptosis besides increasing DNA synthesis in Caco-2 cells (Street *et al.* 2002).

IL-1 β and IL-6 modified the secretion of IGFBP-2 and IGFBP-4 in both the undifferentiated and the differentiated state.

IL-1 β was more effective than IL-6 in both states of differentiation with a significant reduction being observed using physiological concentrations (1 ng/ml). The effect was dose dependent and a significant effect was attained at 1 ng/ml in the undifferentiated state, and at 10 ng/ml in the differentiated state on both IGFBPs. This could be due to a different expression of the IL-1 receptors on the cell surface on days 4 and 14.

IL-6 required higher concentrations to observe a significant reduction in band intensities at both states of differentiation.

IL-1 β and IL-6 had an additive effect as a greater reduction in both IGFBPs was observed when they were given in combination at the same dose. This effect was greater in the undifferentiated state on IGFBP-2. On this IGFBP, the effect of 1 ng/ml of both cytokines was similar to that observed with 50 ng/ml of IL-1 β and IL-6 alone. On IGFBP-4, the effect of both cytokines in combination at 10 ng/ml was similar to that of 50 ng/ml of IL-1 β .

Similarly, in the differentiated state, the effect of both cytokines in combination on IGFBP-2 was greater than that of the interleukins alone, whereas on IGFBP-4, the effects of the cytokines used alone and in combination were similar.

The additive effect of IL-1 β and IL-6 possibly resembles the *in vivo* situation where these two cytokines act synergistically, probably related to the observation that IL-1 β can induce the secretion of IL-6 (Parikh *et al.* 1997).

IGF-I determined a significant reduction of both IGFBP-2 and IGFBP-4 in both states of differentiation. This effect was used as a control as previous studies had shown an effect of this peptide on Caco-2 cells (Hoefflich *et al.* 1996a, 1998, Jehle *et al.* 1999).

The effect of cytokines on IGFBP secretion has been shown to be tissue specific. For example, in human articular chondrocytes *in vitro* (Olney *et al.* 1995), IL-1 had no effect on IGFBP-2 or IGFBP-4, whereas its administration into the central nervous system of adult rats causes a marked increase in IGFBP-2 mRNA (Wood *et al.* 1995). Our data suggest that IL-1 and IL-6 could alter IGFBP secretion in human enterocytes.

IL-1 β and IL-6 are involved in the pathogenesis of CIBD. Both cytokines are increased in the serum and intestinal mucosa of affected subjects (Mahida *et al.* 1989, Brynskov *et al.* 1992, Pullman *et al.* 1992, Stevens *et al.* 1992, Reimund *et al.* 1996). The results of our study would be in favour of a dysregulation of the IGF-IGFBP system in the intestinal mucosa in the presence of increased concentrations of interleukins. A reduction in IGFBP-2 and IGFBP-4 in response to changes in local cytokine concentrations could alter the process of maturation of the enterocyte moving from the crypt to the apex of the villi, as changes in IGFBP-2 and IGFBP-4 would modify IGF-I and IGF-II bioavailability. Hoefflich *et al.* (1998) have shown that in HT-29 cells and in other colonic cancer epithelial cell lines that exogenous

IGFBP-2 inhibits cell proliferation. Thus, it can be speculated that both IL-1 β and IL-6, by lowering further IGFBP-2 and IGFBP-4, could increase the proliferation rate. This mechanism would be in accordance with reports of increased proliferation rate of colon cells in ulcerative colitis and Crohn's disease (Bleiberg *et al.* 1970, Eastwood & Trier 1973, Serafini *et al.* 1981). Furthermore, as proposed by Gibson *et al.* (1995), increased cell proliferation could be dependent on accelerated differentiation and subsequently accelerated cell death. Our recent data would suggest, however, no effect on differentiation and rather increased proliferation and inhibition apoptosis, increasing cell survival (Street *et al.* 2002). These data would suggest that any factor reducing IGFBP-2 and IGFBP-4 could contribute to maintaining the cells in a more 'immature' state.

In conclusion, IL-1 β and IL-6 decreased IGFBP-2 and IGFBP-4 secretion by Caco-2 cells *in vitro* compared with baseline conditions. Further studies are needed to elucidate the mechanisms responsible for these alterations *in vitro*, and to clarify the physiology of cell maturation under circumstances of normal and increased cytokine concentrations.

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