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Interleukin-8 production by the human colon epithelial cell line HT-29: modulation by interleukin-13

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1 We have determined which cytokines induce and modulate the production of the chemokine interleukin-8 (IL-8) by the human colonic epithelial cell line HT-29.

2 Growth arrested cell cultures were stimulated with the human recombinant cytokines interleukin-1α (IL-1α), tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-13 (IL-13), interleukin-10 (IL-10) or vehicle added alone or in combination. The production of IL-8 was determined by enzyme-linked immunosorbent assay (ELISA) and IL-8 messenger RNA expression by Northern blot analysis.

3 The production of IL-8 in unstimulated cells was undetectable by both ELISA and Northern blot analysis.

4 HT-29 cells produced IL-8 following stimulation with IL-1α or TNF-α in a time- and a concentration-dependent manner, while IFN-γ, IL-10 and IL-13 did not induce IL-8 production by HT-29 cells.

5 IL-13 was found to up-regulate significantly ($P<0.01$) the IL-1α but not the TNF-α-induced IL-8 generation by HT-29 cells. In contrast, IL-10 had no effect on either IL-1α or TNF-α-induced IL-8 production.

6 Experiments using cycloheximide demonstrated that this synergistic effect of IL-13 and IL-1α on IL-8 secretion was not through *de novo* protein synthesis. Using actinomycin-D, we demonstrated that the IL-13 up-regulation was at the level of transcription rather than messenger RNA stability.

7 These findings suggest that colonic epithelial cells have a functional IL-13 receptor, which is coupled to an up-regulation of IL-1α, but not TNF-α induced IL-8 generation.

Keywords: Chemokines; interleukin-8; interleukin-13; colon epithelial cells; HT-29; inflammatory bowel disease

Introduction

The intestinal epithelium represents an important interface between the host and external environment serving both as a surface for absorption and a defence against ingested pathogens (McKay & Perdue, 1993a, b). A role for colonic epithelial cells in inflammatory and immune reactions is increasingly recognized. These cells present antigens via class II molecule expression (Mayer *et al.*, 1991; Lowes *et al.*, 1992), express adhesion proteins such as intracellular adhesion molecule-1 (Kaiserlian *et al.*, 1991) and generate soluble inflammatory mediators e.g. arachidonic acid derivatives (Gustafson & Tagesson, 1990; Dias *et al.*, 1992), platelet activating factor (Ferraris *et al.*, 1993), and cytokines (Spriggs *et al.*, 1988; Hedges *et al.*, 1992; Eckmann *et al.*, 1993; Schuerer-Maly *et al.*, 1994; Gross *et al.*, 1995), all of which contribute to the communication between inflammatory cells and cells of the immune system (Sartor, 1994). Several proinflammatory mediators such as interleukin-1 (IL-1), interleukin-6 (IL-6), leukotrienes, tumour necrosis factor-α (TNF-α), prostaglandins, and complement are increased in actively inflamed colonic tissues from patients with inflammatory bowel disease (Lichtman & Sartor, 1993). Levels of mRNA and protein for IFN-γ are increased in Crohn's disease but not in ulcerative colitis (Sartor, 1994; Kolios & Nakos, 1995). Intra-epithelial leukocytes have been reported in intestinal mucosa and these are a prominent characteristic of intestinal inflammation. Although their role remains uncertain it is probable that they are involved in a spectrum of immunological events within intestinal mucosa (Cerf-Benussan & Gay-Grand, 1991; Croitoru & Ernest, 1992). Neutrophil and T- lymphocyte accumulation in lamina propria and infiltration of the colonic epithelium by these cells is a characteristic feature of the intestinal inflammation (Cerf-Benussan & Gay-Grand, 1991; Croitoru & Ernest, 1992). Activated colonic epithelial cell lines have been reported to be a source of the potent neutrophil and T-lymphocyte chemoattractant cytokine IL-8 (Eckmann *et al.*, 1993; Schuerer-Maly *et al.*, 1994; Gross *et al.*, 1995), and an enhanced synthesis of IL-8 has been shown in the mucosa from patients with ulcerative colitis and Crohn's disease (Mahida *et al.*, 1992; Izzo *et al.*, 1993; Mitsuyama *et al.*, 1994; Gibson & Rosella, 1995). Thus colonic epithelial cells have an active role in the recruitment of cells during the inflammatory response.

In the development of an inflammatory response it has become increasingly clear that cytokine networks develop, which allow either amplification of the response or production of a negative feedback signal. Examples of the latter are the modulatory effects of the cytokines interleukin-10 (IL-10) and interleukin-13 (IL-13) on cytokine/chemokine production by activated monocytes and macrophages. Two types of helper T-lymphocytes (Th1 and Th2) were originally defined by Mosmann *et al.* (1986) according to the pattern of cytokines they secreted (Mosmann *et al.*, 1986). Human interleukin 13 was originally identified by screening cDNA libraries from activated peripheral blood lymphocytes (Minty *et al.*, 1993; McKenzie *et al.*, 1993), in the mouse it is expressed by Th2 cells, while in man it can be expressed by both Th1 and Th2 cells (Zurawski & De Vries, 1994). IL-13 is a potent suppressor of cytokine and chemokine generation by activated monocytes and macrophages (Minty *et al.*, 1993; McKenzie *et al.*, 1993; De Waal Malefyt *et al.*, 1993; Zurawski & De Vries, 1994). Interleukin 10 is produced by a variety of cells, including in man Th1 and Th2 cells, and is also a potent suppressor of cytokine/chemokine generation by activated monocytes and

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macrophages (Hsu *et al.*, 1990; Vieira *et al.*, 1991; de Waal Malefyt *et al.*, 1991; Fiorentino *et al.*, 1991; Moore *et al.*, 1993). In addition, polymorphonuclear leukocyte-derived chemokine expression is inhibited by IL-10 (Kasama *et al.*, 1994). The role of IL-10 as negative regulator of colonic inflammation is supported by 'chronic enterocolitis' phenotype expressed in IL-10 deficient mice (Kühn *et al.*, 1993). Furthermore, increased tissue concentrations of IL-10 have been found in ulcerative colitis but not in patients with Crohn's disease (Sartor, 1994). Indeed the activities of IL-10 and IL-13 have led to the suggestion that they may be considered as anti-inflammatory cytokines. We have explored this hypothesis by examining the effect of IL-10 and IL-13 on chemokine expression by activated HT-29 cells, a colonic adenocarcinoma epithelial cell line.

Methods

Cell cultures

HT-29 cell line was purchased from European Collection of Animal Cell Cultures (ECACC). HT-29 cells are a well characterized epithelial cell line derived from a primary colon tumour, which have characteristics of normal intestinal epithelium such as epithelial polarity, presence of the actin-binding protein villin and the occurrence of an enterocytic differentiation (Chantret *et al.*, 1988). Cell cultures were maintained in McCoy's 5A medium (Gibco) supplemented by 10% foetal calf serum, penicillin-streptomycin (10 u ml^{-1} and 10 μg ml^{-1}) and fungizone (0.5 μg ml^{-1}), this combination will be referred to as complete medium. The cells were incubated at 37°C in complete medium in an atmosphere of 5% CO_2 and passaged weekly. For experiments HT-29 cells were seeded at $2-3\times10^4/cm^2$ in 6 well plates (Nunc, U.K.) in complete medium and incubated at 37°C in 5% CO_2 until confluent. Twenty-four hours before stimulation the confluent cell cultures were washed and cultured in fresh complete medium without foetal calf serum. Growth arrested cultures were treated with the appropriate concentration of stimuli in medium without foetal calf serum and incubated as above. In all experiments the final volume of culture fluid was 2 ml in each well, which was comprised of medium with the appropriate concentrations of stimuli. Cell number and viability were checked by Trypan blue at the beginning and the end of each experiment, using representative wells. Viability was always greater than 95%.

Enzyme-linked immunosorbent assay (ELISA) for IL-8

Extracellular IL-8 activity of culture supernatants was measured by a double ligand ELISA, as we have previously described (Brown *et al.*, 1991). A mouse monoclonal anti-IL-8 antibody was used to coat the plates (Nunc Maxisorp, Gibco BRL) and a polyclonal goat IL-8 antibody conjugated to alkaline phosphatase was used for detection. The substrate p-nitrophenyl phosphate (Sigma) was dissolved in 10% diethanolamine buffer pH 9.8 to a final concentration of 1 mg ml^{-1}. The reaction was stopped with 50 μl/well of 3 M NaOH when the desired extinction was reached. The sensitivity of the assay was 0.2 ng ml^{-1}. All ELISA samples were measured in triplicate.

Northern blot analysis

Total cellular RNA was isolated as previously described (Strieter *et al.*, 1989; Brown *et al.*, 1991). Briefly, HT-29 monolayers were solubilized in a solution containing 25 mM Tris (pH 8.0), 4 M guanidine isothiocyanate, 0.5% Sarcosyl, and 0.1 M 2-mercaptoethanol. After homogenization, the above suspension was added to an equal volume of 100 mM Tris (pH 8.0), 10 mM EDTA and 1% SDS. The RNA was then extracted with chloroform-phenol (1:1, v:v) and chloroform-isoamyl alcohol (24:1, v:v). The total RNA was alcohol precipitated and the pellet dissolved in diethyl-pyrocarbonate-

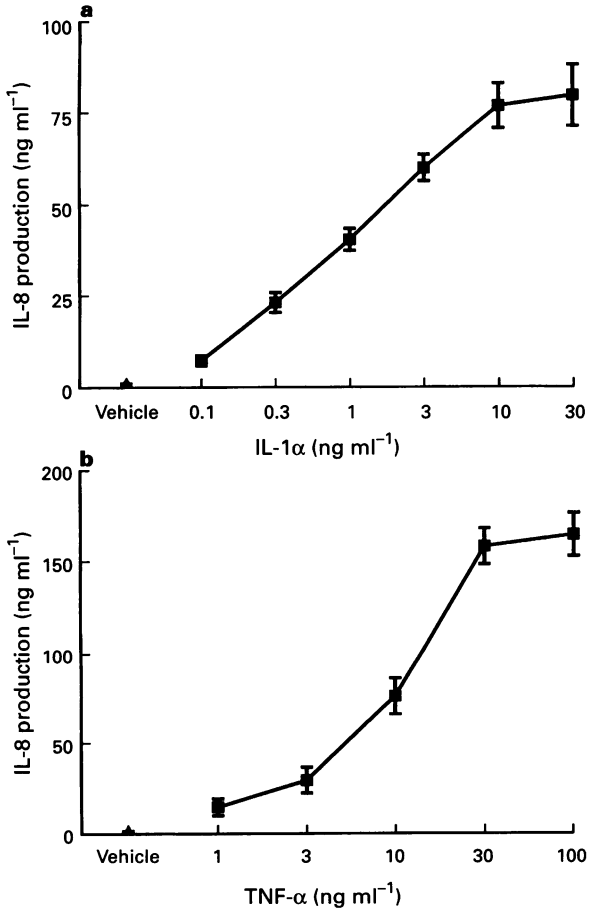

Figure 1 Production of interleukin-8 (IL-8) by HT-29 cells following stimulation for 24 h with IL-1α (a), or tumour necrosis factor-α (TNF-α) (b), or vehicle. Each point is the mean ± s.e.mean of three experiments.

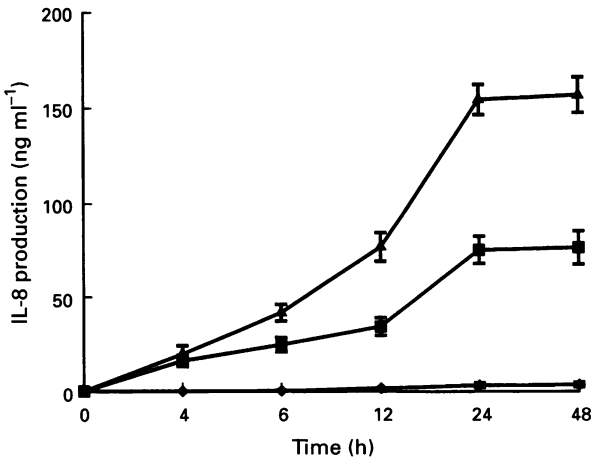

Figure 2 The time course of interleukin-8 (IL-8) production by HT-29 cells following stimulation with IL-1α (■, 30 ng ml^{-1}), or tumour necrosis factor-α (▲, 100 ng ml^{-1}), or interferon-γ (◆, 300 u ml^{-1}). Each point is the mean ± s.e.mean of three experiments.

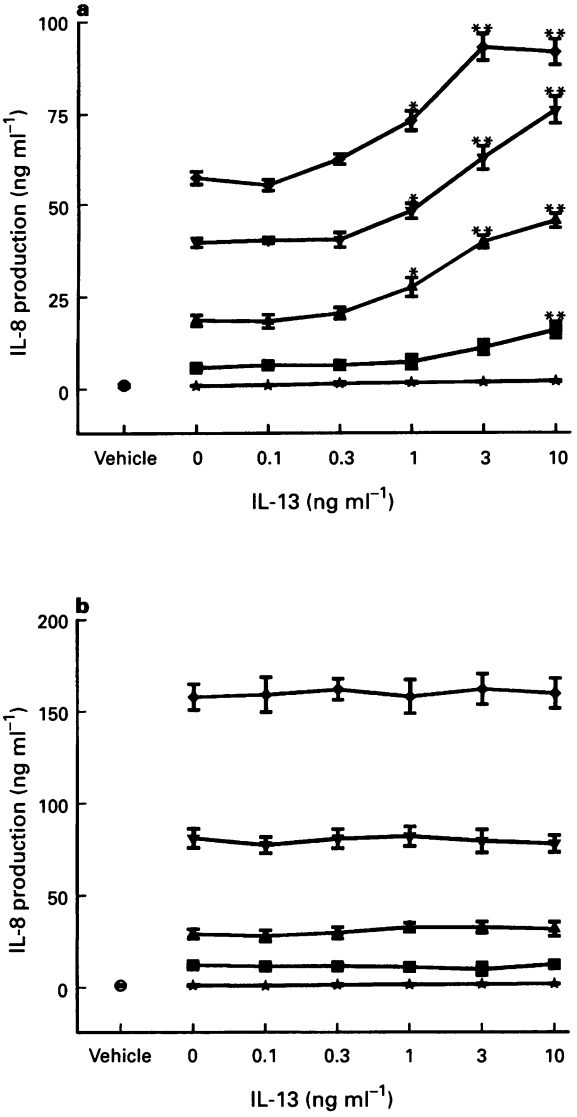

Figure 3 (a) Potentiation of interleukin-1α (IL-1α) (★, 0 ng ml^{-1}; ■, 0.1 ng ml^{-1}; ▲, 0.3 ng ml^{-1}; ▼, 1 ng ml^{-1}; ◆, 3 ng ml^{-1})-induced IL-8 generation by HT-29 cells by increasing concentrations of IL-13 (0.1–10 ng ml^{-1}) after 24 h treatment. (b) Effect of IL-13 (0.1–10 ng ml^{-1}) on tumour necrosis factor-α (★, 0 ng ml^{-1}; ■, 1 ng ml^{-1}, ▲, 3 ng ml^{-1}; ▼ 10 ng ml^{-1}; ◆, 30 ng ml^{-1})-induced IL-8 generation by HT-29 cells after 24 h treatment. Each point is the mean ± s.e.mean of three experiments (*$P<0.05$, **$P<0.01$).

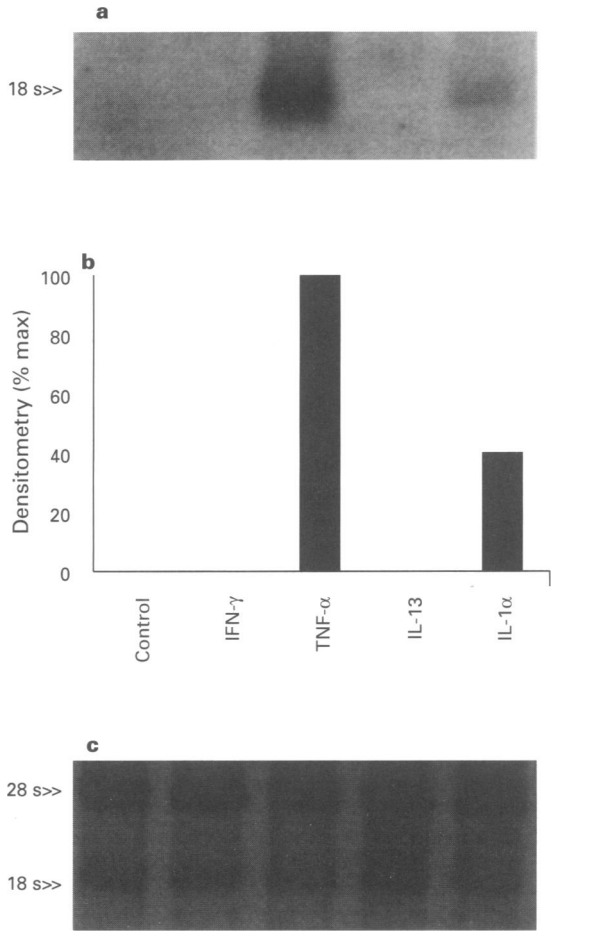

Figure 4 Northern blot analysis of interleukin-8 (IL-8) mRNA expression in HT-29 cells resting or stimulated with vehicle, or interferon-γ (IFN-γ, 300 u ml^{-1}) or tumour necrosis factor-α (TNF-α, 30 ng ml^{-1}) or IL-13 (10 ng ml^{-1}) or IL-1α (3 ng ml^{-1}) for 2 h. (a) The northern blot, (b) densitometry analysis of blot and (c) the ethidium bromide stained 18s and 28s bands indicating equal loading of the lanes. Representative of three experiments.

treated water. The concentration of RNA was measured by obtaining the absorbance at 260 and 280 nm, and 10 μg of RNA was loaded into each well of the agarose gel. Total RNA was separated by electrophoresis using formaldehyde, 1% agarose gels and transferred overnight to nylon membrane (Boehringer Mannheim) by capillary blotting. The blots were baked at 120°C for 20 min and then hybridized as described by Boehringer Mannheim in their DIG luminescent-detection kit. Briefly, membranes were prehybridized for 1 h at 42°C and then hybridized overnight at the same temperature with DIG-labelled oligonucleotide probes (10 ng ml^{-1}) for IL-8 and β-actin. Bound probes were detected with anti-DIG Fab fragments conjugated to alkaline phosphatase with lumigen PPD (Boehringer Mannheim) as the chemiluminescent substrate. Blots were exposed after hybridization to X-ray film. Quantitation of specific chemokine mRNA was performed by laser densitometry. Equivalent amounts of total RNA load per gel lane were assessed by monitoring 18s and 28s RNA.

Materials

Human recombinant IL-1α (specific activity 5×10^7 u mg^{-1}) and TNF-α (specific activity 6×10^7 u mg^{-1}) were generous gifts from Glaxo (Greenford, U.K.) and Bayer (Slough, U.K.), respectively. Human recombinant IFN-γ (specificity $>2.0\times10^7$ u mg^{-1}) was purchased from Boehringer Mannheim, U.K. Human recombinant IL-10 (specific activity 1×10^7 u mg^{-1}) was kindly donated by Dr K.W. Moore (DNAX Palo Alto, California U.S.A.). Human recombinant IL-13 was purified from culture supernatants of stable transfected chinese hamster ovary cells (Minty *et al.*, 1993). Actinomycin-D was purchased from Sigma Chemical and prepared in dimethyl-sulphoxide (DMSO). Cycloheximide and DMSO were also purchased from Sigma Chemical. All cell culture reagents and plastics were from Gibco BRL and Nunc Maxisorp, respectively. The Digoxigenin (DIG) chemiluminescent detection kit for Northern blotting was from Boehringer Mannheim (Lewes, U.K.). 5′-Digoxigenin labelled probes for IL-8 and β-actin were cocktails containing 3 and 4 antisense 30-mer oligonucleotides, respectively, purchased from R & D Systems (Abingdon, U.K.). Antibodies for IL-8 enzyme-linked immunosorbent assay (ELISA) were a generous gift from Dr I.J.D. Lindley (Sandoz Forschungsinstitut, Vienna, Austria).

Statistical analysis

Statistical significance between groups was assessed by 2-way ANOVA followed by Dunnett's test for multiple comparisons to controls. Data are expressed as means ± s.e.mean of three experiments. Triplicate determinations were performed in each experiment. A probability value of $\leqslant 0.05$ was taken as the criterion for a significant difference.

Results

IL-8 secretion from HT-29 cell line in response to cytokines

Confluent monolayers of HT-29 cells were treated with either IL-1α (0.1–30 ng ml^{-1}), or TNF-α (1–100 ng ml^{-1}), IFN-γ (10–300 u ml^{-1}), IL-13 (0.1–10 ng ml^{-1}) or IL-10 (0.1–10 ng ml^{-1}) for 24 h. Supernatants were collected and IL-8 secretion was measured by ELISA. Unstimulated cells did not produce detectable levels of IL-8 (below 200 pg ml^{-1}). The generation of IL-8 was found to be markedly up-regulated in a concentration-dependent manner by either IL-1α or TNF-α (Figure 1a,b). Interestingly, the maximal concentration of TNF-α produced twice as much IL-8 (164.4 ± 6.9 ng ml^{-1}) as was induced by the maximal concentration of IL-1α (79.6 ± 8.4 ng ml^{-1}). Furthermore, neither IFN-γ (Figure 2) nor IL-13 (Figure 3a,b) nor IL-10 (data not shown) stimulated IL-8 generation, when added alone to HT-29 cells. Finally, both IL-1α (30 ng ml^{-1}) and TNF-α (100 ng ml^{-1}) induced a time-dependent generation of IL-8 with significant amounts detected by 4 h and peak production with each cytokine was at 24 h (Figure 2).

Synergistic effect of IL-13 and IL-1α on IL-8 secretion

Possible modulatory effects of IL-10 and IL-13 on IL-1α or TNF-α-induced IL-8 production were examined. Confluent monolayers of HT-29 cells, after 1 h pretreatment with either IL-13 (0.1–10 ng ml^{-1}) or IL-10 (0.1–10 ng ml^{-1}), were treated with either IL-1α or TNF-α. Cells were incubated for 24 h and then IL-8 secretion was measured in supernatants by ELISA. At all concentrations of IL-1α examined, IL-13 (1–10 ng ml^{-1}) produced a significant enhancement of IL-8 generation ($P<0.05$–$P<0.01$) (Figure 3a), for example the presence of IL-13 (10 ng ml^{-1}) induced approximately twice as much IL-8 (91.5 ± 3.5 ng ml^{-1}) compared to that produced by IL-1α (3 ng ml^{-1}) alone (57.1 ± 1.8 ng ml^{-1}, $P<0.01$) (Figure 3a). In marked contrast the same concentration range of IL-13 had no effect on TNF-α induced IL-8 generation (Figure 3b). Furthermore, IL-10 (0.1–10 ng ml^{-1}, $n=3$) was found to have no effect on either IL-1α or TNF-α induced IL-8 production (data not shown).

IL-8 mRNA expression in HT-29 cell line

The time course of IL-8 mRNA expression in epithelial cells was examined. In unstimulated cells no IL-8 transcripts were detected (Figure 4). IL-1α induced IL-8 mRNA expression peaked at 1 h and declined rapidly to a very low expression by 24 h (Figure 5a). In contrast, TNF-α-induced IL-8 mRNA peaked at the same time, but remained high for at least 12 h (Figure 5b). In addition, mRNA expression was not detected after stimulation of the cells with either IFN-γ or IL-13 (Figure 4). To determine whether the synergistic effect of IL-13 with IL-1α on IL-8 generation was at the mRNA level, cells were pretreated for 1 h with IL-13 (5 ng ml^{-1}), then IL-1α (3 ng ml^{-1}) was added and mRNA expression was measured from 1 to 12 h post IL-1α addition. IL-13 was found to prolong significantly the IL-1α-induced mRNA expression in HT-29 cells (Figure 6), while it was without effect on TNF-α-induced IL-8 mRNA expression (data not shown).

Experiments using the protein synthesis inhibitor cycloheximide were then conducted to determine whether the IL-13-induced prolongation of IL-8 mRNA was due to *de novo* protein synthesis. HT-29 monolayers were treated with cycloheximide (5 μg ml^{-1}) for 2 h and then IL-1α (3 ng ml^{-1}) was added alone or after 1 h pretreatment with IL-13 (5 ng ml^{-1}); total RNA was extracted at 1, 2, 4, 6 and 12 h post IL-1α addition. The addition of cycloheximide was without effect on

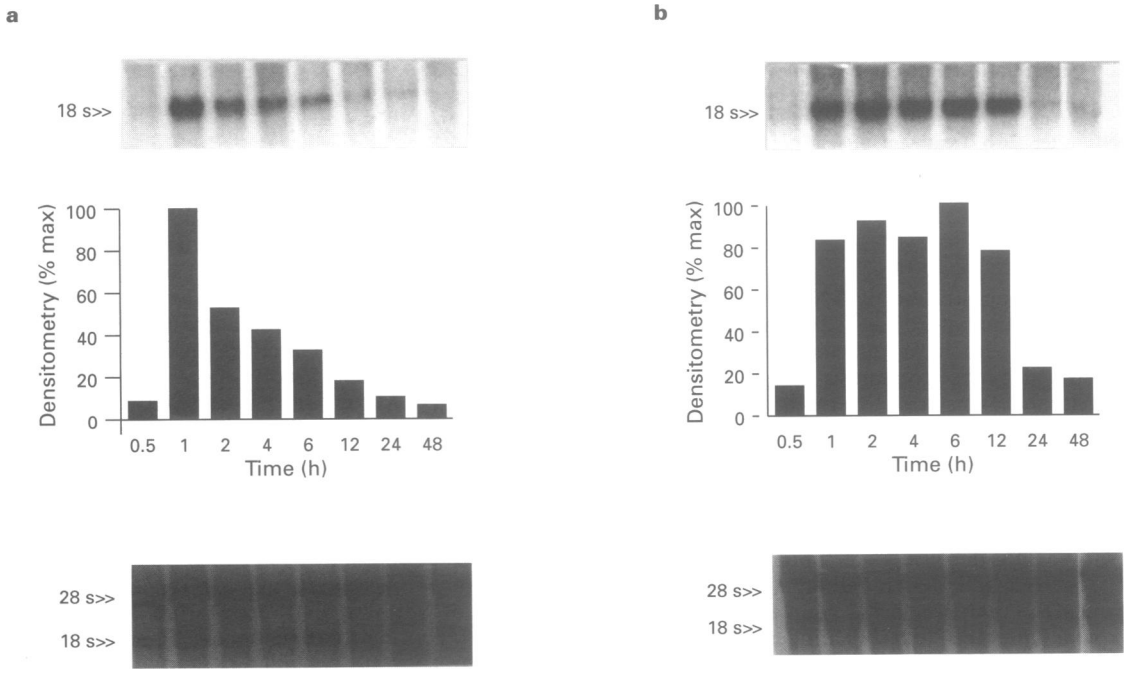

Figure 5 Northern blot analysis of time course of interleukin-8 (IL-8) mRNA expression in HT-29 cells stimulated with (a) IL-1α (3 ng ml^{-1}), or (b) tumour necrosis factor-α (TNF-α, 30 ng ml^{-1}). The top panel of each figure is the northern blot, middle panel is densitometry analysis of blot and lower panel is the ethidium bromide stained 18s and 28s bands indicating equal loading of the lanes. Representative of three experiments.

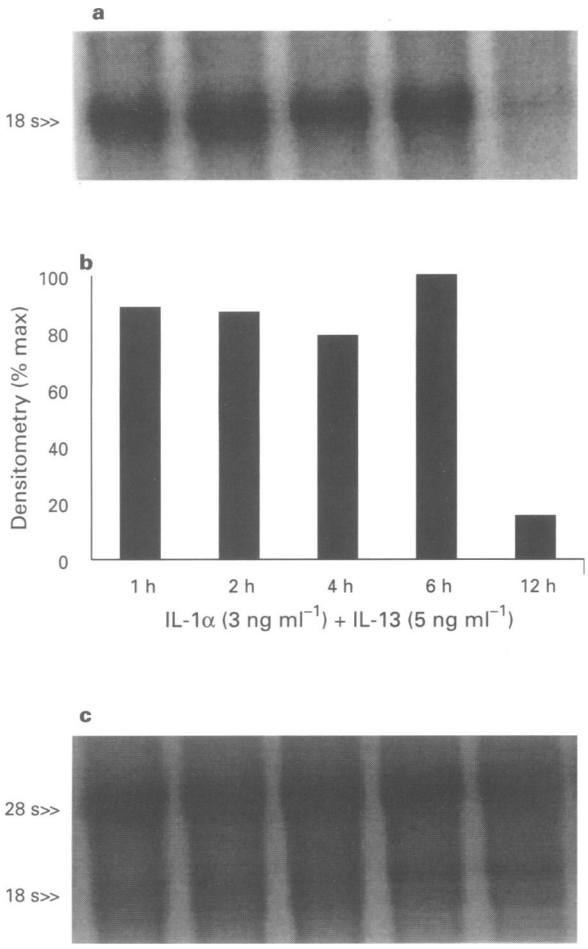

Figure 6 Effect of interleukin-13 (IL-13) on IL-1α induced IL-8 mRNA expression by HT-29 cell line. Cells were pretreated for 1 h with IL-13 (5 ng ml^{-1}) and then IL-1α (3 ng ml^{-1}) was added. Total RNA was extracted at 1, 2, 4, 6 and 12 h and IL-8 mRNA was analysed by Northern blotting. (a) The Northern blot, (b) densitometry analysis of blot and (c) the ethidium bromide stained 18s and 28s bands indicating equal loading of the lanes. Representative of three experiments.

the IL-13-induced prolongation of IL-1α stimulated IL-8 mRNA expression (Figure 7a). Although a small prolongation of IL-1α-induced IL-8 mRNA was produced by cycloheximide (Figure 7b), this prolongation was much weaker compared to that induced by IL-13 (Figure 6).

The above findings demonstrate that IL-13 potentiates the IL-1α-derived IL-8 expression in colonic epithelial cells. The expression of most cytokine mRNA is regulated at the transcriptional or at the mRNA stability level. To determine the mechanism of IL-13-induced regulation, we examined the effect of IL-13 on the $t_{1/2}$ of IL-8 mRNA derived from IL-1α stimulated HT-29. After 1 h incubation with IL-1α (3 ng ml^{-1}) in the presence (1 h pretreatment) of IL-13 (5 ng ml^{-1}) or vehicle, actinomycin-D (5 μg ml^{-1}) was added to the cultures to block further transcription and total RNA was extracted at specific time points. The expression of β-actin mRNA is constitutive, thus its expression is not blocked by actinomycin-D. Analysis of the time course of the IL-8 mRNA/β-actin mRNA ratios in the presence of actinomycin-D provides a measure of the stability ($t_{1/2}$) of IL-1α-induced IL-8 mRNA independent of transcription. Comparison of the IL-8 mRNA/β-actin mRNA ratio in the presence or absence of IL-13 followed by the addition of actinomycin-D demonstrated that IL-13 did not affect IL-8 mRNA $t_{1/2}$ (Figures 8a,b and 9). Therefore the site of increased IL-1α-induced IL-8 mRNA expression in the presence of IL-13 (Figure 6) is likely to be at the level of transcription.

Discussion

In our study, we have demonstrated that IL-13 modulates chemokine expression by colonic epithelial cells. Thus epithelial cells must now be considered together with monocytes, endothelial cells, polymorphonuclear granulocytes and keratinocytes as targets for IL-13 (Herbert *et al.*, 1993; Zurawski & De Vries, 1994; Derocq *et al.*, 1994; Sironi *et al.*, 1994; Colotta *et al.*, 1994). This cytokine produced a doubling in the IL-8 secreted by epithelial cells that were stimulated by IL-1α, but was without effect on cells stimulated by TNF-α. The action of IL-13 on epithelial cells is in marked contrast to the effect of IL-13 on monocytes, in which it is a potent inhibitor of cytokine and chemokine production (Minty *et al.*, 1993; McKenzie *et al.*, 1993; De Waal Malefyt *et al.*, 1993; Zurawski & De Vries, 1994). The inhibitory effect of IL-13 on stimulated chemokine production by monocytes is probably via both the enhanced production of IL-1 receptor antagonists (IL-1ra) and soluble receptors, as well as a direct inhibition of chemokine transcription (Muzio *et al.*, 1994). Thus, when considering cytokine networks, particularly with respect to chemokine production, IL-13 can have a bifunctional role, depending upon the stimulus and the cellular source of chemokines.

It was apparent at both the level of mRNA and peptide that the effect of IL-13 on chemokine production was restricted to IL-1α stimulated cells, as it was without effect on TNF-α stimulated cells. In addressing this unique action of IL-13 a number of observations are worth noting. Firstly, IL-8 production by HT-29 cells by supra-maximal concentrations of TNF-α was approximately twice as much as that produced by supra-maximal concentrations of IL-1α, while stimulation with IFN-γ did not induce a significant production of IL-8 by HT-29 cells, results similar to those obtained previously (Schuerer-Maly *et al.*, 1994). The relative potencies of IL-1α and TNF-α were the reverse of the pattern found in other cells and cell lines (Brown *et al.*, 1991; 1994). Secondly, the differential action of IL-1α and TNF-α on chemokine production was also reflected at the level of IL-8 mRNA. Induction of IL-8 mRNA with IL-1α peaked at 1 h, then rapidly subsided, in contrast, TNF-α induced IL-8 mRNA expression similarly peaked by 1 h, but was maintained for at least 12 h. Thirdly, IL-13 caused a prolongation of IL-1α induced IL-8 mRNA expression that was similar to that present in TNF-α stimulated cells. Fourthly, experiments with cycloheximide, a protein synthesis inhibitor, demonstrated that the effect of IL-13 on IL-8 mRNA expression did not depend upon protein synthesis. Thus the action of IL-13 on epithelial cells is unlikely to involve IL-1ra because this would produce an inhibition of IL-1α action not an enhancement, and the involvement of IL-1ra is not consistent with the lack of effect of cycloheximide on IL-8 mRNA. Finally, experiments with actinomycin-D indicate that IL-13 produces an increase in IL-1α-induced IL-8 mRNA at the level of transcription rather than by affecting mRNA stability.

Interleukin-10 is a 35 kD homodimeric cytokine, which was originally described as a Th2-cell-derived cytokine. Recent studies have shown that IL-10 is produced by a variety of cells, including Th2 cells, Ly-1 B cells, mast cells and cells of the macrophage lineage (Moore *et al.*, 1993). This cytokine at concentrations of 10 ng ml^{-1} or less is an effective inhibitor of cytokine generation by IFN-γ and/or LPS stimulated human monocytes (De Waal Malefyt *et al.*, 1991), prostaglandin E_2 production by IL-1 or LPS activated human monocytes (Poole *et al.*, 1995) and chemokine generation by polymorphonuclear leukocytes treated with LPS (Kasama *et al.*, 1994). Thus IL-10 is considered to be a modulator of the inflammatory response. However, we found that IL-10 throughout the concentration range of 0.1 to 10 ng ml^{-1} had no effect on either IL-1α or TNF-α-induced IL-8 production by the colonic epithelial cell

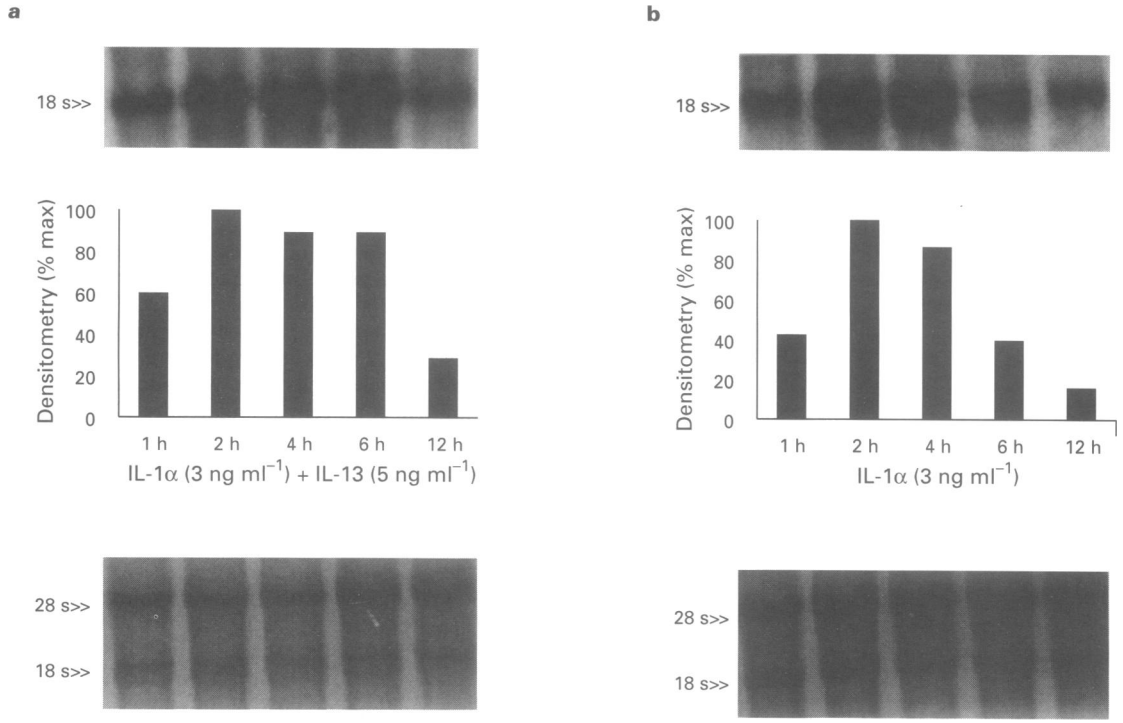

Figure 7 (a) Effect of interleukin-13 (IL-13) on IL-1α induced IL-8 mRNA expression in HT-29 cells in the presence of cycloheximide. After 1 h pretreatment with cycloheximide (5 $\mu g\,ml^{-1}$), IL-13 (5 $ng\,ml^{-1}$) was added and the incubation continued for 1 h. Then IL-1α (3 $ng\,ml^{-1}$) was added. (b) Effect of cycloheximide (5 $\mu g\,ml^{-1}$) on IL-1α induced IL-8 mRNA expression in HT-29 cells. Cells were pretreated for 2 h with cycloheximide (5 $\mu g\,ml^{-1}$) and then IL-1α (3 $ng\,ml^{-1}$) was added. Total RNA was extracted at 1, 2, 4, 6 and 12 h and IL-8 mRNA was analysed by Northern blotting. Representative of three experiments.

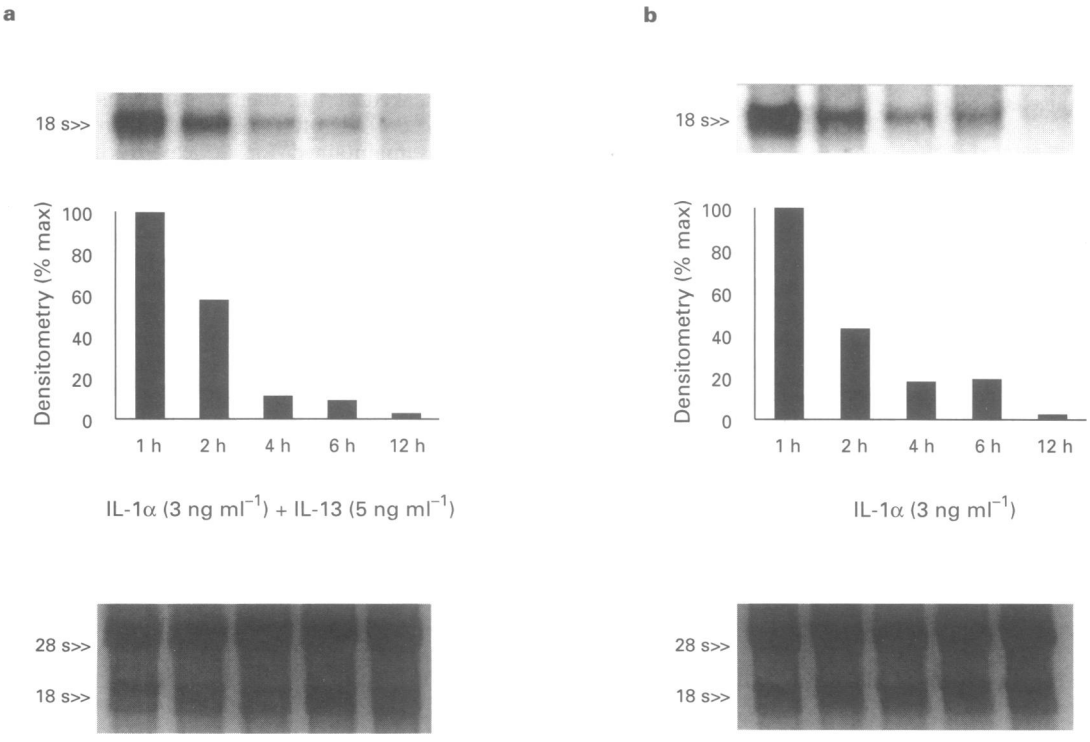

Figure 8 (a) Effect of interleukin-13 (IL-13) on IL-1α induced IL-8 mRNA expression in HT-29 cells in the presence of actinomycin-D (5 $\mu g\,ml^{-1}$) added after 1 h treatment with IL-1α (3 $ng\,ml^{-1}$) and IL-13 (5 $ng\,ml^{-1}$). (b) IL-1α induced IL-8 mRNA expression in HT-29 cells in the presence of actinomycin-D (5 $\mu g\,ml^{-1}$) added after 1 h treatment with IL-1α. Total RNA was extracted at 1, 2, 4, 6 and 12 h and IL-8 mRNA was analysed by Northern blotting. Representative of two experiments.

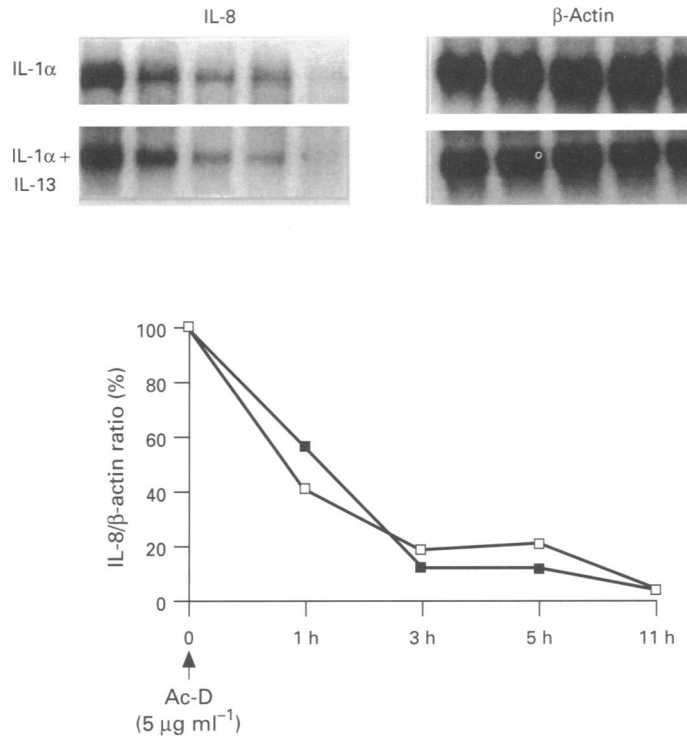

Figure 9 The $t_{1/2}$ of interleukin-8 (IL-8) mRNA determined 1 h after stimulation with IL-1α. Cells were stimulated for 1 h with IL-1α (3 ng ml^{-1}) in the absence (□) or presence (■) of IL-13 (5 ng ml^{-1}). Then, actinomycin-D (5 μg ml^{-1}) was added and the decay of IL-8 mRNA was assessed by Northern blot analysis. The $t_{1/2}$ of IL-1α-induced IL-8 mRNA in the presence or absence of IL-13 were 1.05 h and 0.9 h, respectively. Representative of two experiments.

line HT-29. Therefore the expression of 'chronic enterocolitis' phenotype in IL-10 deficient mice (Kühn *et al.*, 1993) is probably not due to the deficiency of a direct suppressive action of IL-10 on colonic epithelial cells.

The intestinal epithelial cells constitute a barrier between the environment and the host tissue and they are the first cells to come in contact with many pathogens. From this position intestinal epithelial cells might play a crucial role as an outpost of the immune system located in the underlying intestinal mucosa; soluble mediators produced by these cells might function as an early signal to neighbouring immune cells and be involved in the recruitment of cells during the inflammatory response. The transmigration of selected populations of leukocytes is a multiple-step process and requires a series of coordinated signals, which include the expression and activation of adhesion molecules as well as the generation of a leukocyte specific chemotactic gradient by the cells of the extravascular component (Carlos & Harlan, 1994). However, the nature of the stimulus and the subsequent spectrum of chemotactic factors produced, determine the specific leukocyte population elicited to the inflammatory site (Baggiolini *et al.*, 1994). This hypothesis is supported by the characterization of a large family of target cell-specific chemotactic polypeptides, now collectively known as chemokines (Lindley *et al.*, 1993a,b). The members of this family are generally less than 10 kD, they are basic heparin-binding polypeptides and belong either to the C-X-C family of which the neutrophil chemoattractant IL-8 is the best characterized, or the C-C subfamily of which the monocyte-chemotactic peptide (MCP-1) is the prototype (Miller & Krangel, 1992). Production of the potent neutrophil and T-lymphocyte chemoattractant cytokine IL-8 from intestinal epithelial cells may have a crucial effect on intra-epithelial and lamina propria neutrophils, and lymphocytes during the intestinal inflammation. The infiltration of T-cells induced by IL-8 could be particularly relevant to the role of colonic epithelial cells as antigen presenting cells (Mayer *et al.*, 1991).

Thus, this capacity of colonic epithelial cells to secrete IL-8, might be important in the acute inflammatory response, as neutrophil infiltration of the intestinal mucosa is a characteristic feature of intestinal inflammation and an increased production of IL-8 has been reported in colonic mucosa from patients with ulcerative colitis and Crohn's disease (Mahida *et al.*, 1992; Izzo *et al.*, 1993; Mitsuyama *et al.*, 1994). In addition, the findings that IL-1α and TNF-α, cytokines released by activated macrophages during an acute inflammatory response, induce IL-8 production by colonic epithelial cells suggest a communication between these cells and immune cells located in the underlying intestinal mucosa. Furthermore, our results demonstrating that the Th2-lymphocyte-derived cytokine IL-13 synergizes with IL-1α in IL-8 secretion and expression by colonic epithelial cells, support the view that there is an interaction between epithelial cells and lymphocytes in the initiation and maintenance of the intestinal inflammation.

It will be important to determine whether isolated epithelial cells from normal individuals and from patients with inflammatory bowel disease also respond to the same stimuli. This might provide useful information on the leukocyte recruitment in the colonic epithelium during intestinal inflammation and help with the understanding of the pathogenesis of inflammatory bowel disease.

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