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GM-CSF, IL-1α, IL-1β, IL-6, IL-8, IL-10, ICAM-1 and VCAM-1 gene expression and cytokine production in human duodenal fibroblasts stimulated with lipopolysaccharide, IL-1α and TNF-α

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SUMMARY

The role of mucosal fibroblasts in intestinal inflammatory reactions is not known. In this study, we demonstrate that fibroblasts grown from histologically normal human duodenal biopsy tissues expressed mRNA genes for granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1α, IL-1β, IL-6, IL-8, IL-10, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) when stimulated with lipopolysaccharide (LPS) or IL-1α. The increased mRNA expression of GM-CSF, IL-1α, IL-1β, IL-6 and IL-8 in response to IL-1α and LPS stimulation was time- and dose-dependent. In contrast, IL-10 was weakly expressed when fibroblasts were stimulated with LPS, IL-1α or tumour necrosis factor-alpha (TNF-α), but the expression was enhanced in the presence of cycloheximide combined with optimal concentrations of LPS, IL-1α or TNF-α. IL-1α was a more potent stimulator than LPS for GM-CSF, IL-6, IL-8 and IL-10 expression, but not for IL-1α and IL-1β. Increased GM-CSF, IL-6 and IL-8 gene expression was associated with the production of cytokine proteins in culture supernatant, but IL-1α and IL-1β remained undetectable. Dexamethasone suppressed both gene expression and protein production of GM-CSF, IL-6 and IL-8 when fibroblasts were exposed to IL-1α. TNF-α stimulated the release of GM-CSF, IL-6 and IL-8 and, combined with IL-1α, cytokine production was enhanced synergistically. Finally, both LPS and IL-1α up-regulated ICAM-1 and VCAM-1 gene expression. These findings implicate duodenal fibroblasts in the initiation and/or regulation of intestinal inflammation.

Keywords cytokines lipopolysaccharide duodenal fibroblasts ICAM-1 VCAM-1

INTRODUCTION

Fibroblasts have been shown to be important effector cells in the inflammatory response. Skin, synovial and pulmonary fibroblasts produce granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), leukaemia inhibitory factor (LIF), IL-1, IL-6 and IL-8 when stimulated with IL-1 or tumour necrosis factor (TNF) [1–5]. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are expressed on the surface of fibroblasts [6,7]. Cytokine secretion is increased by bacterial lipopolysaccharide (LPS) and viruses [8,9], suggesting a role in host defence. Furthermore, studies with respiratory and synovial tissues have demonstrated that growth factors and cytokines released from cultured fibroblasts can support the growth, differentiation and activation of inflammatory cells, including primed T cells [3,10]. These observations imply that, following direct stimulation by pathogens or indirect stimulation by inflammatory cytokines in response to infection, production of cytokines and the expression of adhesion molecules by activated fibroblasts at an inflammatory site play a role in the amplification and regulation of inflammation.

The role of the inflammatory response in determining functional and structural mucosal abnormalities in intestinal disease remains unclear. Elevated levels of IL-1β, G-CSF, IL-6 and transforming growth factor-beta (TGF-β) have been detected in the intestinal lamina propria of patients with inflammatory bowel disease possibly secreted by mononuclear cells [11,12]. Increased ICAM-1 expression by lamina propria phagocytes has been reported in both inflammatory bowel disease and coeliac disease [13,14]. The role of small intestinal fibroblasts has not, however, been studied with respect to cytokine secretion and the expression of adhesion molecules. This study examines the effects of IL-1, TNF and LPS on gene expression of GM-CSF, IL-1α, IL-1β, IL-6 and IL-8, and the adhesion molecules, ICAM-1 and VCAM-1, in fibroblasts cultured from histologically normal duodenal biopsy tissues. In

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addition, the expression of IL-10, a pleiotropic cytokine implicated in the regulation of immune and inflammatory reactions [15], is also investigated. The findings indicate that fibroblasts participate in a novel pathway of immunoregulation, and are well placed to modulate the level of inflammation in gut mucosal tissue in both normal and diseased state.

PATIENTS AND METHODS

Subjects

Duodenal biopsies of normal histology were obtained at endoscopy from three subjects (all females, age 23–48 years) undergoing investigation for diarrhoea. Tissue was immediately placed in Dulbecco's modified Eagle's medium (DMEM; Commonwealth Serum Lab., Melbourne, Australia) containing streptomycin, penicillin, gentamycin and 10% fetal calf serum (FCS). This study was approved by the Hunter Area Health and University Human Ethics Committees.

Tissue culture

The biopsy tissue (5–10 mg) was finely minced with a scalpel. The tissue fragments were then resuspended in 2 ml DMEM containing 10% FCS, L-glutamine (2·5 mM), 2-mercaptoethanol (1×10^{-5} M) and antibiotics, and seeded in 200-μl aliquots into wells of a 96-well round-bottomed microtitre plate. After 24 h the culture supernatants from each well were removed and replaced with fresh medium. This procedure was repeated every 2–3 days until fibroblast outgrowth occurred. Cultures with positive growth were treated with trypsin/versene solution after removal of culture medium. The cells were collected, pooled and washed by centrifugation before transferring to a 24-well culture plate (Corning, Miami, FL) and allowed to grow to confluency. One hundred per cent of cultured cells were fibroblasts as verified by immunoperoxidase staining using mouse monoclonal anti-human fibroblast (Dako fibroblast 5B5; Dakopatt A/S, Glostrup, Denmark). Before use, fibroblasts were cultured in duplicates in complete DMEM medium for 2–3 days until confluent in a 24-well tissue culture plate. The cells were stimulated with various concentrations of *Escherichia coli* 0111: B4 LPS (Sigma-Aldrich, Sydney, Australia), human recombinant IL-1α or TNF-α (Boehringer-Mannheim, Sydney, Australia) for specified times. The culture supernatants were removed for cytokine assays, and the adherent cells, recovered by treatment with trypsin/versene solution, were counted and pelleted by centrifugation for mRNA extraction.

Isolation of mRNA

Twenty thousand fibroblasts were lysed in SDS buffer containing RNase and Proteinase-K (GIBCO-BRL, Melbourne, Australia), incubated at 45°C, and then absorbed with oligo $(dT)_{20}$ cellulose (GIBCO-BRL). The cellulose was washed in a spin-column with binding buffer (Tris-HCl pH 7·6, 0·5 M NaCl, 1 mM EDTA, 0·1% SDS) followed by a low salt buffer wash. The poly A^+ RNA was then eluted with elution buffer, precipitated with ethanol containing glycogen and 2 M sodium acetate, and recovered by centrifugation (16000 *g*) for 15 min. The ethanol was removed and the mRNA pellet was resuspended in 9·5 μl of DEPC water.

Reverse transcription-polymerase chain reaction

cDNA synthesis and amplification using reverse transcription-polymerase chain reaction (RT-PCR] reactions were carried out as previously described [16]. Briefly, cDNA synthesis was carried out at 42°C for 1 h in 30 μl final volume containing: (i) 3 μl of MMLV-RT (200 U/ml); (ii) 1·0 μl of RNasin (40 U/ml); (iii) 6·0 μl of 5 × MMLV-RT buffer (250 mM Tris-HCl, 375 mM KCl, 15 mM $MgCl_2$); (iv) 3·0 μl of dithiothreitol (0·1 M); (v) 3·0 μl of Oligo $d(T)_{15}$ (0·5 ng/ml); (vi) 3·0 μl of acetylated bovine serum albumin (BSA; 1 mg/ml) and 1·5 μl of dNTP (10 mM each). Five microlitres of first strand cDNA were added to PCR mix containing: 0·2 μl Taq DNA polymerase (50 U/ml; Promega Corp., Rozelle, Australia); 1·2 μl 25 mM $MgCl_2$; 2 μl 10 × PCR buffer; 1 μl 4 mM dNTP mix; 8·6 μl-sterile distilled water; 1 μl 20 μM anti-sense primer; and 1 μl 20 μM sense primer. The mixture was subjected to amplification using a thermal cycler (Pharmacia LKB, Uppsala, Sweden) set at 94°C for 1 min, 57°C for 2 min and 72°C for 3 min for 25–35 cycles. PCR fragments were separated on ethidium bromide-stained 2% agarose gel (Nusieve) and then blotted onto Hybond nylon membrane (Amersham, Aylesbury, UK) for Southern blot analysis with oligoprobes. Hybridization was carried out using ECL-labelling and detection kits (Amersham). Blots were exposed on a blue light-sensitive Hyperfilm MP (Amersham) to detect light output.

Primers and oligoprobes

Primers and oligoprobes for GM-CSF, IL-1α, IL-1β, IL-6 and IL-8 were purchased from Clontech (Palo Alto, CA). The following forward and reverse primers and oligoprobes for VCAM-1, ICAM-1 and IL-10 were synthesized from published DNA sequences [17–19] by Bresatec (Adelaide, Australia):

ICAM-1	Primers	5′ AGA ACC TTA CCC TAC GCT GC 3′ (443–462)
		5′ CAG TAT TAC TGC ACA CGT CAG C 3′ (939–918)
	Probe	5′ GCT GGC AGG ACA AAG GTC TGG AGC TGG TAG (704–675)
VCAM-1	Primers	5′ GGA AGT GGA ATT AAT TAT CCA A 3′ (1599–1622)
		5′ CTA CAC TTT TGA TTT CTG TG 3′ (2040–2021)
	Probe	5′ GAT TCA CAT TCA TAT ACT CCC GCA TCC TTC 3′ (1839–1810)
IL-10	Primers	5′ CTG TGA AAA ACA AGA GCA AGG C 3′ (432–443)
		5′ GAA GCT TCT GTT GGC TCC C 3′ (922–904)
	Probe	5′ CGA CTC ATA GAC TCT AGG ACA TAA 3′ (582–607)

Quantification of cytokine in culture supernatants

GM-CSF and IL-8 were measured by ELISA kits (Amersham). The sensitivity of this assay is 1·5 pg GM-CSF/ml and 4·4 pg IL-8/ml. IL-6 activity in the culture supernatant was assessed by a proliferative assay using B9 cells [20]. Briefly, 50 μl of two-fold serial dilutions of standard human IL-6 in DMEM or culture supernatants were dispensed into wells of a flat-bottomed

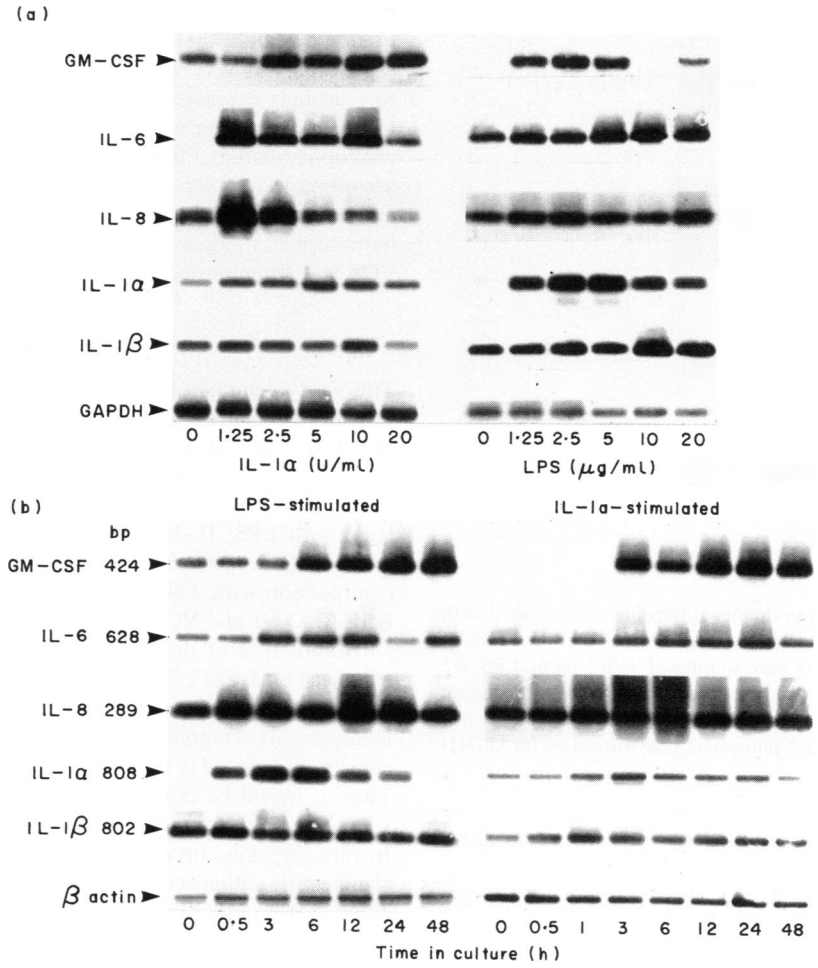

Fig. 1. Effects of IL-1α and lipopolysaccharide (LPS) concentrations on granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1α, IL-1β, IL-6 and IL-8 gene expression in duodenal fibroblasts. (a) Duodenal fibroblasts were stimulated for 24 h with different concentrations of IL-1α or LPS. (b) Duodenal fibroblasts were stimulated with 5 μg/ml LPS or 2·5 U/ml IL-1α for 0, 0·5, 1, 3, 6, 12, 24 and 48 h. Equivalent loading of each sample was determined by β actin message shown below. The arrows indicate the expected sizes (bp) of the polymerase chain reaction (PCR) products for GM-CSF, IL-1α, IL-1β, IL-6 and IL-8. Equivalent loading of each sample was verified by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) message shown below.

microtitre plate (Nunc, Roskilde, Denmark). To each well, 50 μl of B9 cells ($2·5 \times 10^4$/ml) in DMEM were added. Cell proliferation was assessed by adding 10 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan, 5 mg/ml; Sigma Aldrich, Sydney, Australia) to each well. After incubation for 4 h, isopropanol (100 μl) was added to lyse the cells. The plates were read at 590 nm on an ELISA plate reader.

RESULTS

GM-CSF, IL-1α, IL-1β, IL-6 and IL-8 gene expression in duodenal fibroblasts stimulated with LPS and IL-1α

To determine the level of GM-CSF, IL-1α, IL-β, IL-6 and IL-8 mRNA gene expression, fibroblasts were cultured with or without IL-1α or LPS. As shown in Fig. 1a, without stimulation the signals for cytokine gene expression were variable, from low to undetectable, but when stimulated were significantly up-regulated in a dose-dependent manner. Different doses of IL-1α and LPS were required for maximal expression of different cytokines. IL-1α induced a stronger response for GM-CSF, IL-6 and IL-8, whereas LPS induced a stronger IL-1α and IL-1β expression. Similar results were obtained with two other fibroblast lines (data not shown). Increased expression of cytokine mRNA genes was time-dependent (Fig. 1b). With the exception of GM-CSF, cytokine expression peaked between 6 and 24 h after stimulation, followed by a fall at 24–48 h.

IL-10 gene expression enhanced by cycloheximide in duodenal fibroblasts stimulated with LPS, IL-1α and TNF-α

IL-10 was not constitutively expressed by duodenal fibroblasts, but it was weakly expressed when stimulated with optimal concentrations of LPS, IL-1α or TNF-α (Fig. 2). However, in the presence of cycloheximide, a protein synthesis inhibitor, the signal for IL-10 expression was greatly enhanced. Superinduction of GM-CSF, IL-6 and IL-8 was also observed when fibroblasts were stimulated with LPS, IL-1α or TNF-α combined with cycloheximide (data not shown).

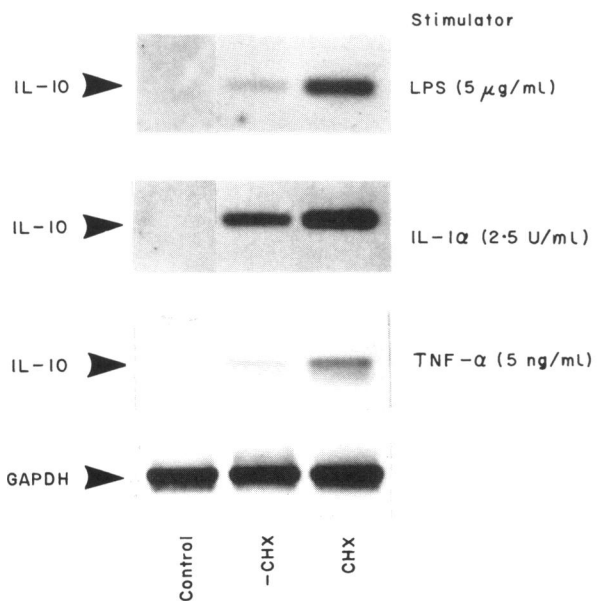

Fig. 2. IL-10 gene expression in duodenal fibroblasts stimulated with lipopolysaccharide (LPS), IL-1α and tumour necrosis factor-alpha (TNF-α). Duodenal fibroblasts were stimulated with 5 μg/ml LPS, 2·5 U/ml IL-1α or 5 ng/ml TNF-α in the absence or presence of 5 μg/ml cycloheximide (CHX) for 24 h. Equivalent loading of each sample was determined by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) message shown at the bottom.

Cytokine protein production by IL-1α- and LPS-stimulated duodenal fibroblasts

Table 1 shows cytokine protein production in three fibroblast lines 24 h after stimulation with IL-1α and LPS. However, IL-1α and IL-1β were undetectable (data not shown). Depending on the cytokine and the cell line assayed, LPS-stimulated fibroblasts produced approximately 2–40 times as much cytokine as unstimulated controls. The induction of cytokine production was significantly enhanced when stimulated with IL-1α instead of LPS. Cytokine synthesis was inhibited in the presence of cycloheximide or actinomycin-D (data not shown).

Effects of dexamethasone on cytokine gene expression and protein production

Incubation of fibroblasts with IL-1α and various concentrations of dexamethasone for 10 h lowered the production of cytokine proteins in a dose-related manner (Fig. 3). Fibroblasts treated in identical fashion also expressed decreased levels of GM-CSF, IL-6 and IL-8 mRNAs, and this down-regulation was particularly apparent for the expression of GM-CSF (Fig. 3).

Synergistic stimulation of cytokine production by TNF-α and IL-1α

Figure 4 shows that stimulation of fibroblasts with TNF-α resulted in the production of GM-CSF, IL-6 and IL-8 in a dose-dependent manner. In terms of the amount of cytokine secreted, IL-8 was more sensitive to TNF-α stimulation than GM-CSF or IL-6. However, in combination with IL-1α, the production of all three cytokines was significantly enhanced compared with cells stimulated with TNF-α alone. The order of sensitivity to various concentrations of TNF-α in combination with IL-1α was IL-8 > IL-6 > GM-CSF.

ICAM-1 and VCAM-1 gene expression in duodenal fibroblasts

Figure 5 shows that, in unstimulated fibroblasts, ICAM-1 expression was very weak to undetectable, whereas VCAM-1 was strongly expressed. When stimulated with LPS or IL-1α, ICAM-1 expression was significantly enhanced and VCAM-1 was up-regulated. Furthermore, expression of genes for adhesion molecules was associated with the production of these molecules on the surface of fibroblasts, as detected by immunofluorescence staining using mouse MoAbs to ICAM-1 and VCAM-1 (Immunotech, Marseilles, France) (data not shown).

DISCUSSION

This study demonstrates that fibroblasts cultured from histologically normal duodenal biopsies express GM-CSF, IL-1α, IL-1β, IL-6 and IL-8 mRNA genes which can be up-regulated in a time- and dose-related manner when exposed to LPS or IL-1α. Duodenal fibroblasts also expressed IL-10 when stimulated with LPS, IL-1α or TNF-α. Cytokine production was greatly enhanced when fibroblasts were stimulated with IL-1α in combination with TNF-α. Lastly, LPS or IL-1α up-regulated both ICAM-1 and VCAM-1 gene expression.

Stimulation of duodenal fibroblasts with LPS and IL-1α enhanced GM-CSF, IL-1α, IL-1β, IL-6, IL-8 and IL-10 mRNA gene expression, indicating that these cells participate in the inflammatory response within the gut mucosa. The gut is constantly exposed to lumenal antigens including bacterial LPS. Thus, it would be expected that under normal circumstances, LPS would induce cytokine expression in intestinal fibroblasts. In turn, cytokine production by duodenal fibroblasts may be significantly enhanced by cytokines such as IL-1 and TNF released from other mucosal cells.

It is not known whether LPS responsiveness of duodenal fibroblasts represents a phenotype that is characteristic of the tissue organ. Recent studies have demonstrated that fibroblast heterogeneity exists [9,21] and that, in respiratory tissues, lung fibroblasts are insensitive to LPS, unlike nasal fibroblasts [9].

Although stimulated duodenal fibroblasts expressed IL-1α and IL-1β mRNA genes, the secreted products were not detected. It is possible, however, that IL-1 proteins involved in immune and inflammatory responses are membrane-bound, as recent studies have shown that LPS- and IL-1-stimulated human fibroblasts contained cell-associated thymocyte-stimulating activity [22,23]. Our findings also demonstrated that duodenal fibroblasts can produce IL-6, and this production can be up-regulated by IL-1α, TNF-α or LPS. IL-6 induces the production of metalloproteinases inhibitor in fibroblasts [24], it provides a second signal for T cell activation [25], and it promotes growth and differentiation of B cells [26], suggesting a potential role for IL-6 in the fibrotic response during chronic inflammation and in local immune responses. The release and accumulation of IL-8 contributes to the recruitment of neutrophils, while GM-CSF modulates and maintains inflammation through the promotion of growth and differentiation, as well as activating various cell types [3,27]. Taken together, our findings identify fibroblasts as key intermediate cells, with a role in the maintenance, regulation, and direction of inflammation within the gut mucosa.

In this study, dexamethasone was shown to suppress gene expression and production of GM-CSF, IL-6, and IL-8 in fibroblasts stimulated with IL-1α. This is consistent with other

Table 1. Cytokine production by duodenal fibroblast lines

	GM-CSF (pg/ml)			IL-6 (ng/ml)			IL-8 (ng/ml)		
Cell lines	—	IL-1α	LPS	—	IL-1α	LPS	—	IL-1α	LPS
1	140	4100	310	2·57	147	16·6	2·36	37	9·6
2	120	2700	240	4·20	270	22	3·2	238	11·6
3	160	1000	245	1·0	66	38	16	60	41

Three different duodenal fibroblast lines were cultured to confluency in 24-well plates and then stimulated in the absence or presence of 5 U/ml IL-1α or 10 μg/ml *Escherichia coli* 0111:B4 LPS for 24 h. The culture supernatants were collected and assayed for cytokine production. Each value represents the pool of duplicate cultures.

GM-CSF, Granulocyte-macrophage colony-stimulating factor; LPS, lipopolysaccharide.

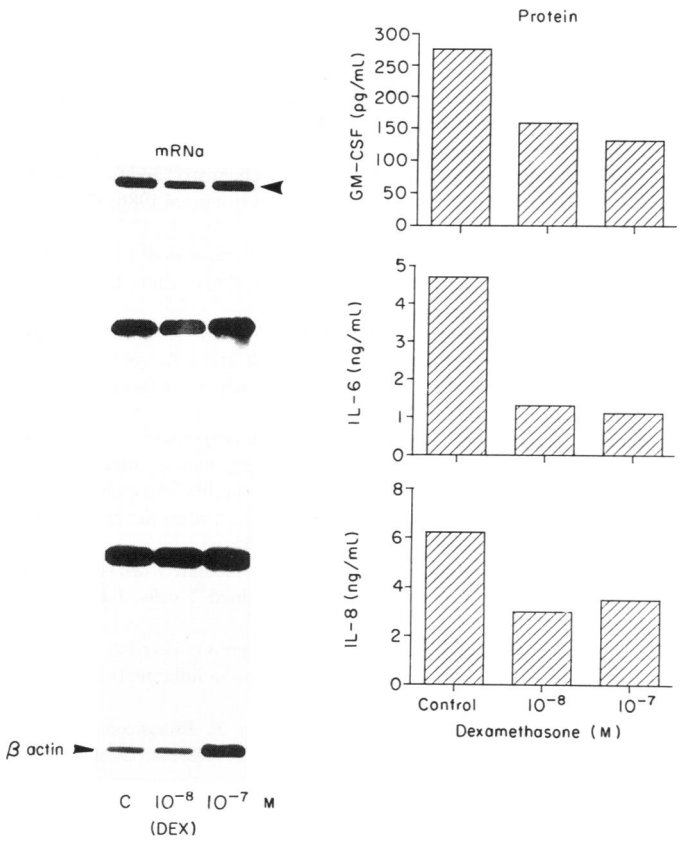

Fig. 3. Effects of dexamethasone on granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6 and IL-8 gene expression and cytokine production in duodenal fibroblasts. Duodenal fibroblasts were stimulated with 2·5 U/ml IL-1α in the absence or presence of dexamethasone (DEX) at different concentrations for 12 h. Each value for GM-CSF, IL-6 and IL-8 protein production represents the pool of duplicate cultures. Equivalent loading of each sample was verified by β actin message shown below.

studies where dexamethasone suppressed cytokine-mediated up-regulation in nasal [9] and skin [28] fibroblasts, as well as inhibiting protein production. However, our studies show that there was a differential response to this inhibitory effect, as GM-CSF was more sensitive at the level both of gene expression and of protein production compared with IL-6 and IL-8.

IL-10 is produced by many cells, including T cells, B cells, monocytes and mast cells. It has a range of biological activities, including the down-regulation of T cell proliferation, cytokine production and macrophage-accessory cell functions [29,30], and the promotion of mast cell growth and survival [31]. Our studies show that the IL-10 gene transcript is not constitutively expressed in unstimulated duodenal fibroblasts, but it is expressed when stimulated with IL-1α, or to a lesser extent with LPS or TNF-α. However, when stimulated in the presence of cycloheximide the signal was enhanced, probably due to increased stability of cytokine mRNA [32]. Nevertheless, the expression of IL-10 in duodenal fibroblasts indicates that an environment rich in IL-10 would favour the development of Th2-like cells within the gut mucosa.

Recent studies demonstrated that TNF-α synergizes with IL-1α or interferon-gamma (IFN-γ) in regulating IL-6 production in human lung fibroblasts [33]. Our demonstration that TNF-α interacts with IL-1α to augment GM-CSF, IL-6 and IL-8 production in a synergistic fashion is consistent with this observation. In addition, IL-1α or TNF-α in combination with LPS also induced increased cytokine production in a similar manner (unpublished data), suggesting that IL-1α, TNF-α and LPS acting in synergy may contribute to amplification of the inflammatory response *in vivo*.

Our studies demonstrated that VCAM-1, but not ICAM-1, was strongly expressed in duodenal fibroblasts. Following exposure to IL-1 or LPS, VCAM-1 gene expression was up-regulated, while that of ICAM-1 was enhanced. Thus, duodenal fibroblasts may play a role in mediating cellular interactions in local immunity by binding to immune and inflammatory cells via adhesion receptors such as the integrins LFA-1 and VLA-4. Recent studies have demonstrated that ICAM-1 expression is up-regulated in cells of the small intestinal lamina propria from patients with coeliac disease, but the cell types involved were not defined [13,34]. Several studies have failed to detect ICAM-1 on epithelial cells *in situ*, but this adhesion molecule appears to be expressed in colonic carcinoma cell lines [35]. This is the first report of the expression of VCAM-1 and ICAM-1 on intestinal fibroblasts. In addition, IL-1α and TNF-α released by local cells may up-regulate adhesion molecules on fibroblasts, and thus facilitate leucocyte attachment with subsequent shaping of the inflammatory response.

In conclusion, (i) LPS, IL-1α, and TNF-α stimulate cytokine gene expression in duodenal fibroblasts; (ii) the same stimuli induce cytokine protein production; (iii) IL-1α and TNF-α

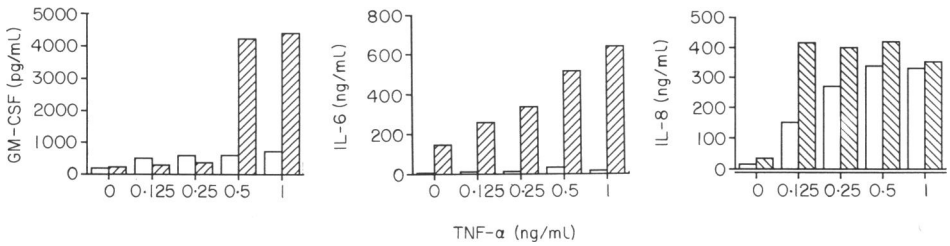

Fig. 4. Synergistic stimulation of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6 and IL-8 production by IL-1α and tumour necrosis factor-alpha (TNF-α). Duodenal fibroblasts were stimulated with TNF-α at different concentrations alone or in the absence or presence of 2·5 U/ml IL-1α for 24 h. Each value represents the pool of duplicate cultures. □, −IL-1; ■, +IL-1.

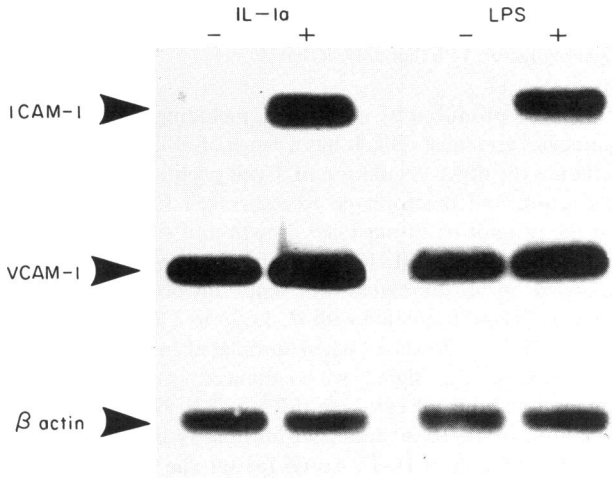

Fig. 5. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) gene expression in duodenal fibroblasts stimulated with IL-1α and lipopolysaccharide (LPS). Fibroblasts were stimulated with 2·5 U/ml IL-1α or 5 μg/ml LPS for 6 h. Equivalent loading of each sample was verified by β actin message shown at the bottom.

stimulate enhanced cytokine production synergistically; and (iv) LPS, IL-1α and TNF-α stimulate the expression of adhesion molecules. These findings imply that duodenal fibroblasts may contribute to the regulation of inflammatory reactions in the gut.

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REFERENCES

1 Fibbe WE, Van Damme J, Billiau AN *et al.* Human fibroblasts produce granulocyte-CSF, macrophage-CSF, and granulocyte-macrophage-CSF following stimulation by interleukin-1 and poly(r-I)·poly(rC). Blood 1988; **72**:860–6.

2 Leizer T, Cebon J, Layton JE *et al.* Cytokine regulation of colony-stimulating factor production in cultured human synovial fibroblasts. I. Induction of GM-CSF and G-CSF production by interleukin-1 and tumor necrosis factor. Blood 1990; **76**:1989–96.

3 Vancheri C, Ohtoshi T, Cox G *et al.* Neutrophilic differentiation induced by human upper airway fibroblast-derived granulocyte/macrophage colony-stimulating factor (GM-CSF). Am J Respir Cell Mol Biol 1991; **4**:11–17.

4 Rolfe M, Kunkel S, Standiford T *et al.* Pulmonary fibroblast expression of interleukin-8: a model for alveolar macrophage-derived cytokine networking. Am J Respir Cell Mol Biol 1991; **5**:493–501.

5 Hamilton JD, Waring PM, Filonzi EL. Induction of leukemia inhibitory factor in human synovial fibroblasts by IL-1 and tumor necrosis factor. J Immunol 1993; **150**:1496–502.

6 Dustin ML, Rothlein R, Bahn AK *et al.* Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 1986; **137**:245–54.

7 Wilkinson L, Edwards J, Poston R *et al.* Expression of vascular cell adhesion molecule-1 in normal and inflamed synovium. Lab Invest 1993; **68**:82–93.

8 Damme JV, Schaafsma MR, Fibbe WE *et al.* Simultaneous production of interleukin 6, interferon-β and colony-stimulating activity by fibroblasts after viral and bacterial infection. Eur J Immunol 1989; **19**:163–8.

9 Zhou X, Jordana M, Gauldie J. Lipopolysaccharide induces granulocyte-macrophage colony-stimulating factor, interleukin-8, interleukin-6 gene expression in human nasal fibroblasts but not in lung fibroblasts. Evidence for heterogeneity within the respiratory tract. Am J Resp Cell Mol Biol 1993; **9**:255–63.

10 Scott S, Pandolfi F, Kurnick J. Fibroblasts mediate T cell survival: a proposed mechanism for retention of primed T cells. J Exp Med 1990; **172**:1873–6.

11 Stevens S, Walz G, Singaram C *et al.* Tumour necrosis factor-α, interleukin-1β, and interleukin-6 expression in inflammatory bowel disease. Dig Dis Sci 1992; **37**:818–26.

12 Pullman W, Elsbury S, Kobayashi M *et al.* Enhanced mucosal cytokine production in inflammatory bowel disease. Gastroenterology 1992; **102**:529–37.

13 Malizia G, Calabrese A, Cottone M *et al.* Expression of leukocyte adhesion molecules in inflammatory bowel disease. Gastroenterology 1991; **100**:150–9.

14 Strugess R, Macartney J, Makgoba M *et al.* Differential upregulation of intercellular adhesion molecule-1 in coeliac disease. Clin Exp Immunol 1990; **82**:489–92.

15 Moore K, O'Garra A, de Waal Malfety R *et al.* Interleukin-10. Annu Rev Immunol 1993; **11**:165–90.

16 Pang G, Buret A, Batey R *et al.* Morphological, phenotypic and functional characterisation of a pure population of $CD54^+$ $CD16^-$ $CD3^+$ large granular lymphocytes generated from human duodenal mucosa. Immunology 1993; **79**:498–505.

17 Moore K, Vieira P, Fiorentino D *et al.* Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein Barr Virus. Science 1990; **248**:1230–4.

18 Stauton D, Steven D, Stratowa C *et al.* Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and intergrin supergene families. Cell 1988; **52**:925–33.

19 Birdsall H, Lane C, Ramser M *et al.* Induction of VCAM-1 and

ICAM-1 on human neural cells and mechanisms of mononuclear leukocyte adherence. J Immunol 1992; **148**:2717–23.

20 Aarden LA, Lansdorp PM, DeGroot ER. A growth factor for B cell hybridoma produced by human monocytes. Lymphokines 1985; **10**:175–185.

21 Phipps R, Penney D, Keng D *et al.* Immune functions of subpopulations of lung fibroblasts. Immunol Res 1990; **9**:275–86.

22 Elias JA, Freundlich B, Kern JA *et al.* Cytokine networks in the regulation of inflammation and fibrosis in the lung. Chest 1990; **97**:1439–46.

23 Weinstein LJ, Gubler DU, Vilcek J. Induction of membrane associated interleukin-1 by tumour necrosis factor in human fibroblasts. J Immunol 1987; **138**:2137–42.

24 Lotz M, Guerne P. Interleukin-6 induces the synthesis of tissue inhibitor of metalloproteinases-1/erythroid potentiating activity (TIMP-1/EPA). J Biol Chem 1991; **266**:2017–20.

25 Ceuppens J, Baroja M, Lorre K *et al.* Human T cell activation with phytohemagglutinin. The function of IL-6 as an accessory signal. J Immunol 1988; **141**:3868–74.

26 Hirano T, Taga T, Nakano NK *et al.* Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). Proc Natl Acad Sci USA 1985; **85**:5490–4.

27 Jordana M, Cox G, Ohtoshi T *et al.* The TDR concept in chronic airways inflammation: tissue driven response. In: Matsson P, Ahlstedt S, Venge P *et al.*, eds. Clinical impact of the monitoring of allergic inflammation. London: Academic Press 1991:33–46.

28 Helgott D, May L, Sthoeger Z *et al.* Bacterial lipopolysaccharide (endotoxin) enhances expression and secretion of β-2 interferon by human fibroblasts. J Exp Med 1987; **166**:1300–9.

29 Taga K, Tosato G. IL-10 inhibits T cell proliferation and IL-2 production. J Immunol 1992; **148**:1143–8.

30 de Waal Malefyt R, Abrams J, Bennett B *et al.* IL-10 inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; **174**:1209–20.

31 Thompson-Snipes L, Dhar V, Bond M *et al.* Interleukin-10: a novel stimulatory factor for mast cells and their progenitors. J Exp Med 1991; **173**:507–10.

32 Akashi M, Loussararian A, Adelman D *et al.* Role of lymphotoxin in expression of interleukin 6 in human fibroblasts. J Clin Invest 1990; **85**:121–9.

33 Elias JA, Lentz V. IL-1 and tumor necrosis factor synergistically stimulate fibroblast IL-6 production and stabilize IL-6 messenger RNA. J Immunol 1990; **145**:161–6.

34 Smart C, Clabrese A, Oakes D *et al.* Expression of the LFA-1b2 intergrin (CD11a/CD18a) and ICAM-1 (CD54) in normal and coeliac small bowel mucosa. Scand J Immunol 1991; **34**:299–305.

35 Kvale D, Krajci P, Brandtzaeg P. Expression and regulation of adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) in human intestinal epithelial cell lines. Scand J Immunol 1992; **35**:669–76.