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Chemokine Gene Expression and Secretion by Cytokine-Activated Human Microvascular Endothelial Cells

Differential Regulation of Monocyte Chemoattractant Protein-1 and Interleukin-8 in Response to Interferon-γ

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The elicitation of leukocytes from the circulation to inflamed tissue depends on the activation of both the leukocyte and endothelial cell. In this study we determined the gene expression and secretion patterns for the chemokines interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) in cytokine- and lipopolysaccharide (LPS)-treated cultured human lung microvascular endothelial cells (HLE). HLE constitutively expressed low levels of MCP-1 and IL-8. Treatment of HLE with a variety of cytokines and LPS upregulated both IL-8 mRNA expression and release of immunoreactive IL-8 with an order of potency tumor necrosis factor-α (TNF-α) ≫ IL-1α > LPS, whereas interferon-γ (IFN-γ) had no effect on IL-8 mRNA or antigenic levels. However, IFN-γ, in combination with high doses of IL-1α, resulted in a synergistic increase in IL-8 generation. MCP-1 gene expression and secretion was induced in a dose-dependent manner after IL-1α, TNF-α, IFN-γ, and LPS activation of HLE. IL-1α was the most potent inducer of MCP-1 generation and LPS was relatively ineffective. IFN-γ, in combination with low doses of IL-1α, resulted in a synergistic increase in MCP-1 generation by HLE. These results demonstrate that although IL-8 and MCP-1 generation by HLE occurs on cytokine treatment, the relative ability of a given cytokine to elicit IL-8 generation is not directly parallel to effects on MCP-1 generation. These data suggest that the regulation of IL-8 and MCP-1 expression exhibit significant differences in their mechanisms. Such differences in the expression of specific chemokines may explain the specific appearance of various leukocytes at sites of inflammation and injury. These data also directly demonstrate that the lung microvascular endothelium contribute to the cytokine network of the lung, with the ability to respond to locally generated cytokines and to produce potent mediators of the local inflammatory response. (Am J Pathol 1994, 145:913–921)

Recruitment of leukocytes from the circulation to the site of tissue injury is a prominent feature of tissue damage and inflammation. The elicitation of specific leukocyte populations to the inflammatory site is regulated at many levels and requires a series of coordinated signals. Endothelial cells lining the postcapillary venules are the primary site at which extravasation of leukocytes occurs,[1] in the lung, however, the capillaries are the main site of migration. Studies with large vessel-derived endothelial cells have demonstrated that on activation by proinflammatory stimuli, the endothelium can increase the expression of specific adhesion molecules and produce inflammatory cytokines such as interleukin-1 (IL-1),

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IL-8, and monocyte chemoattractant protein-1 (MCP-1).[2,3] Although adhesion of leukocytes to the endothelium is a prerequisite to diapedesis, the migratory response is thought to be directed by a *trans*-endothelial gradient of soluble chemoattractants.[1]

Recently, a family of target cell-specific chemotactic polypeptides now known as chemokines,[4] have been identified and structurally characterized by the location of four cysteine residues.[5] The released peptides of this family are generally less than 10 kd 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 MCP-1 is the prototype.[6] A variety of cells express genes for and produce IL-8 and MCP-1 in response to IL-1 tumor necrosis factor-α (TNF-α), and a wide variety of exogenous agents including lipopolysaccharide (LPS).[5,6] The cell selective chemoattractant properties, cellular sources, and induction by proinflammatory cytokines all suggest that both IL-8 and MCP-1 are important mediators of the local inflammatory response.

The chemokine IL-8 was originally characterized as a potent chemotactic factor for neutrophils but it is also a chemoattractant for basophils and T cells.[7–9] In addition, IL-8 can, depending on the *in vitro* experimental model, either up-regulate[10] or down-regulate[11] neutrophil adhesion to the endothelium. Indeed Huber et al[10] demonstrated that IL-8 is the primary promoter of neutrophil diapedesis by virtue of its ability to both regulate leukocyte-endothelial cell adhesion molecule and β_2-integrin expression and to form a *trans*-endothelial cell chemotactic gradient. In contrast, studies by Gimbrone et al[11] have shown that endothelial-derived IL-8 can function as a neutrophil-directed leukocyte adhesion inhibitor. IL-1α, TNF-α, and LPS treatment of human umbilical vein endothelial cells (HUVEC) results in the release of $[Ala\text{-}IL\text{-}8]_{77}$,[11,12] an NH_2-terminal pentapeptide extended form of $[Ser\text{-}IL\text{-}8]_{72}$, the latter being the predominant molecular species of IL-8. Both forms of IL-8 have qualitatively similar activities in the stimulation of polymorphonuclear neutrophil (PMN) degranulation, inhibition of PMN adhesion to activated endothelium, inhibition of PMN accumulation at inflammatory sites when injected intravascularly, and stimulating PMN accumulation when administered extravascularly.[13,14] Strieter et al[3] demonstrated that TNF-α, LPS, and IL-1 induced gene expression for IL-8 in HUVEC and studies by several other laboratories confirmed these findings again using HUVEC as the cellular model.[12,15]

The gene for the monocyte chemoattractant, MCP-1 is encoded by the human homologue of the platelet-derived growth factor-inducible murine gene JE.[16,17] Several groups have reported that IL-1β and TNF induce the expression of MCP-1/JE mRNA in HUVEC and secrete an immunoreactive form of MCP-1/JE.[18–20]

The principal site of neutrophil adhesion and emigration is not at the level of large conduit veins such as the umbilical vein; it is at the level of the postcapillary venule. There are now numerous studies demonstrating important structural, biochemical, antigenic, and functional differences in endothelial cells from diverse sites.[21,22] Currently, it is not known whether endothelial cells of microvascular origin are capable of producing IL-8 and MCP-1, nor is there any information available relating to cytokine regulation of microvascular endothelial cell chemokine production. To investigate the regulation of IL-8 and MCP-1 in microvascular endothelium, we have used endothelial cells (HLE) derived from human lung microcirculation.[23] We report that HLE can produce IL-8 and MCP-1 in response to inflammatory cytokines, and that the expression of these two chemokines demonstrates differential responsiveness to interferon-γ (IFN-γ).

Materials and Methods

Microvascular Endothelial Cell Preparation and Culture Conditions

HLE were isolated from the peripheral lobes of human lungs and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 10% Nu-serum (Collaborative Research, Bedford, MA), 20 µg/ml heparin, 4 µl/ml retinal-derived growth factor prepared as described by D'Amore et al,[24] 2 mmol/L L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin as previously reported by Carley et al.[23] Pure endothelial cell cultures were obtained by fluorescence-activated cell sorting, based on the uptake of acetyl low density lipoprotein labeled with 1,1′-dioctadecyl-1,3,3,3′3′-tetramethyl-indocarbocyanine perchorate (Dil-Ac-LDL) and characterized as previously described.[23,25] Cells used in this study were passages three to seven after sorting.

Cytokines

Human recombinant TNF-α and IFN-γ both with a specific activity of 2×10^7 U/mg were obtained from Boehringer Mannheim (Indianapolis, IN). Human recombinant IL-1α specific activity of 10^5 U/µg was from

Genzyme (Boston, MA). LPS was purchased from Sigma (St. Louis, MO) serotype 0111:B4.

Northern Blot Analysis

Total cellular RNA from HLE cells was isolated using a modification of the method of Chirgwin et al[26] and Jonas et al[27] and separated by Northern blot analysis using the method described by Strieter et al.[28] Briefly, after cytokine treatment for 6 hours HLE cells were solubilized in a solution consisting of 25 mmol/L Tris, pH 8, containing 4.2 M guanidine isothiocyanate, 0.5% Sarkosyl, and 0.1 M 2-mercaptoethanol. After homogenization, the above suspension was added to an equal volume of 100 mmol/L Tris, pH 8, containing 10 mmol/L EDTA and 1% sodium dodecyl sulfate and the RNA was then extracted with chloroform-phenol and chloroform-isoamyl alcohol. The RNA was alcohol precipitated and the pellet dissolved in 10 mmol/L Tris and 0.1 mmol/L EDTA buffer with 0.1% Sarkosyl. The concentration of RNA was determined by obtaining the absorbance at A_{260} and A_{280} nm and 10 μg of RNA was loaded in to each well of the agarose gel. RNA was analyzed by the Northern blot technique using formaldehyde, 1% agarose gels, and transblotting to nitrocellulose. The blots were baked under vacuum prehybridized, and then hybridized with a ^{32}P 5′-end-labeled oligonucleotide probe. The 30 mer oligonucleotide probes were complementary to either nucleotides 262 to 291 or nucleotides 256 to 285 of published cDNA sequence for IL-8 and MCP-1, respectively.[29,30] The sequence of the IL-8 probe was 5′-GTT-GGC-GCA-GTG-TGG-TCC-ACT-CTC-AAT-CAC-3′,[29] whereas the sequence for the MCP-1 probe was 5′-TTG-GGT-TTG-CTT-GTC-CAG-GTG-GTC-CAT-GGA-3′.[30] Blots were quantitated by laser densitometry.[28] Equivalent amounts of total RNA loaded per gel lane was assessed by monitoring 18S and 28S RNA.

ELISA for IL-8 and MCP-1

Extracellular IL-8 activity of culture supernatants was measured using a double ligand ELISA method, as previously described.[31] The detection limit of this assay was 200 pg/ml. Anti-human IL-8 mouse polyclonal antibody, human recombinant IL-8, and anti-human IL-8 goat polyclonal antibody conjugated to alkaline phosphatase were supplied by Dr. Ivan Lindley of Sandoz, Vienna, Austria. The substrate *p*-nitrophenyl phosphate (Sigma) was dissolved in 10% diethanolamine buffer, pH 9.8, to a final concentration of 1 mg/ml. The reaction was stopped with 50 μl/well of 3 M NaOH when the desired extinction had been reached; absorbance was determined at 405 nm in an ELISA plate reader.

Antigenic MCP-1 in culture supernatants was measured using a double ligand method, as previously described.[32] The detection limit of this assay was 50 pg/ml. The assays were performed using rabbit anti-human MCP-1 antibody, human recombinant MCP-1, biotinylated rabbit anti-human MCP-1, and avidin-horseradish peroxidase (Dako Ltd., Carpinteria, CA). The chromogen substrate in this instance was orthophenylenediamine dichloride in 25 mmol/L citrate/phosphate, pH 5, and 0.0002% hydrogen peroxide and the reaction was terminated with 50 μl/well of 3 M H_2SO_4. The absorbance was read at 490 nm in a ELISA plate reader. The rabbit anti-human MCP-1 antibody showed no cross-reactivity to the following members of the chemokine family: MCP-2, MCP-3, RANTES, and MIP-1α and -β (J. Van Damme, personal communication).

Experimental Protocol

For experiments HLE (10^5 cells/well) were plated on to 24-multiwell tissue culture plates. Twenty-four hours before stimulation confluent cell cultures were washed and cultured in RPMI supplemented with 5% FBS. To treat HLE the cytokines (IL-1α, TNF-α, LPS, IFN-γ, and IL-1 + IFN-γ) were diluted to the required concentrations in RPMI plus 1% FBS. HLE culture medium was then removed and replaced with either an equal volume of medium containing cytokines or medium alone and the cells were incubated for 18 hours at 37 C in 5% CO_2/95% air: after incubation culture supernatants were removed and stored at −85 C for antigenic determination of both IL-8 and MCP-1 by ELISA.

Data Analysis

Antigenic values for IL-8 and MCP-1 are expressed as mean ± SEM (n = 3 to 5/group). Multiple comparisons were analyzed by one-way analysis of variance and if indicated post hoc analysis performed using Bonferroni's modification of Student's *t*-test. The null hypothesis was rejected at $P < 0.05$.

Results

Cytokine-Induced Gene Expression of IL-8 and MCP-1 in HLE

Unstimulated HLE expressed undetectable or relatively low levels of IL-8 (Figure 1) and MCP-1 (Figure

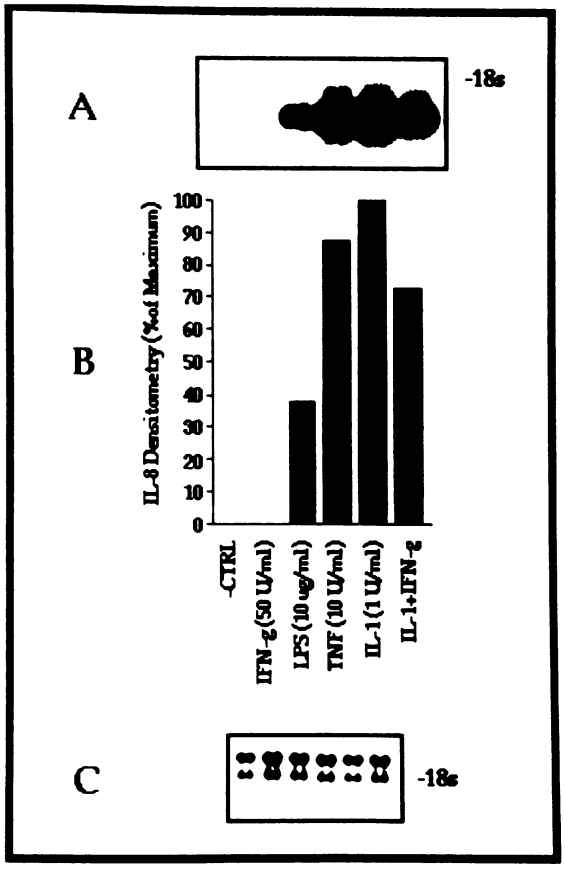

Figure 1. *IL-8 gene expression by cytokine and LPS-activated human lung microvascular endothelial cell.* A: *Represents Northern blot analysis of IL-8 mRNA by HLE cells 6 hours after treatment with either medium alone (Ctrl), IFN-γ, LPS, TNF-α, IL-1α, or IL-1α and IFN-γ. Laser densitometry of each respective Northern blot is represented in* B, *whereas the 18S ribosomal RNA demonstrating equal loading of RNA is shown in* C.

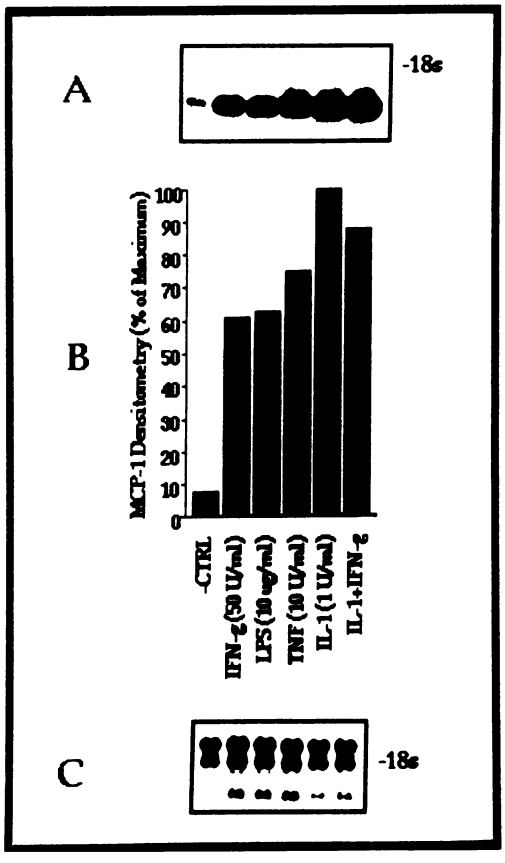

Figure 2. *Cytokine and LPS-activated human lung microvascular endothelial cells express MCP-1 mRNA. HLE cells were stimulated with either medium alone (Crtl), IFN-γ, LPS, TNF-α, IL-1α, or IL-1α and IFN-γ and total RNA was extracted 6 hours after challenge.* A: *Represents Northern blot of the MCP-1 mRNA expression.* B: *Laser densitometry of each respective Northern blot.* C: *18S ribosomal RNA demonstrating equal loading of RNA.*

2) mRNA transcripts. On exposure to IL-1α (1 U/ml), TNF-α (10 U/m), LPS (10 μg/ml), or IL-1 and IFN-γ (1 U/ml + 50 U/ml) for 6 hours high levels of IL-8 mRNA transcripts were detected. Densitometric analysis of Northern blots revealed a 100, 85, 72, and 38% expression of IL-8 mRNA after exposure to IL-1α, TNF-α, IL-1 plus IFN-γ, or LPS, respectively (Figure 1). Interestingly, no IL-8 mRNA transcripts were detected after treatment of HLE with IFN-γ alone (50 U/ml) (Figure 1). Similarly, a good induction of MCP-1 mRNA was observed after treatment of HLE with either IL-1α, TNF-α, LPS, IL-1 plus IFN-γ, or IFN-γ alone resulting in 100, 75, 62, 88, and 61% of MCP-1 transcripts, respectively (Figure 2). Unlike IL-8, however, MCP-1 gene expression was observed in HLE after stimulation with IFN-γ alone (Figure 2). MCP-1 and IL-8 transcripts induced by a combination of IL-1α with IFN-γ were less than the additive effects of IL-1 and IFN-γ alone (Figure 2). Thus, all of the cytokines used induced MCP-1 gene expression in marked contrast to IL-8 expression, which was not induced after treatment with IFN-γ.

Synthesis and Release of Chemotactic Peptides by HLE Cells

Supernatants derived from microvascular endothelial cells cultured in the presence of medium alone contained <200 pg/ml of either IL-8 or MCP-1 peptide. Interestingly, the kinetics for the generation of antigenic IL-8 and MCP-1 after IL-1 stimulation in HLE cells were different. We observed time-dependent activation of IL-8 after IL-1α treatment, IL-8 was detectable from 4 hours, peaks at 18 hours, and then declines, and a 50% decrease in IL-8 production was observed at 48 hours compared with peak IL-8 production at 18 hours (Table 1). In contrast, the kinetics for MCP-1 production are clearly different from that of

Table 1. *Time-Dependent Induction of Antigenic IL-8 and MCP-1 by IL-1α-Stimulated Microvascular Endothelial Cells*

Time (hours)	IL-8 (ng/ml) Mean ± SEM	MCP-1 (ng/ml) Mean ± SEM
0	<0.3	<0.3
1	<0.3	<0.3
4	9.3 ± 0.2*	5.7 ± 0.4*
18	11.9 ± 0.6*	38.0 ± 3.5*
24	9.2 ± 0.7*	42.0 ± 2.0*
48	5.9 ± 0.3*	48.3 ± 1.66*

* Values significantly different ($P < 0.05$) from time 0 hours for each chemokine. One-way analysis of variance followed by Bonferroni modified *t*-test. Values are mean ± SEM of four separate determinations and represent accumulated levels of IL-8 or MCP-1 at the indicated time points.

IL-8, both chemokines are elevated at 4 hours, however, MCP-1 production continues to rise over the entire time course studied, an eightfold increase of MCP-1 production occurs at 48 hours (Table 1).

Treatment of HLE cells with either IL-1α, TNF-α, or LPS at concentrations ranging from 0.01 to 10 U/ml, 0.02 to 200 U/ml, or 0.1 to 100 μg/ml, respectively, for 18 hours elicited a dose-dependent increase in IL-8 generation with a maximum occurring at 1 U/ml of IL-1α, 20 U/ml of TNF-α, and 1 μg/ml of LPS (Figure 3). Although TNF-α (20 U/ml) induced maximal IL-8 secretion, IL-1α was a more potent inducer of IL-8 generation because 1 U/ml of IL-1α was able to induce equivalent IL-8 generation (Figure 3). IL-8 release was undetectable in HLE cells treated with IFN-γ at concentrations ranging from 0.5 to 500 U/ml (Figure 3). In combination with the higher concentrations of IL-1α (1 to 10 U/ml), IFN-γ synergistically increased IL-8 release compared with IL-1α alone (Figure 3). With the lower dose of IL-1α (0.01 and 0.1 U/ml) IFN-γ co-treatment did not modify the IL-8 production compared with IL-1α alone.

Treatment of HLE for 18 hours with either IL-1α, TNF-α, or IFN-γ resulted in a dose-dependent increase in MCP-1 generation and antigenic induction was maximal at 10 U/ml of IL-1α, 20 U/ml of TNF-α, and 500 U/ml of IFN-γ (Figure 4). In contrast to IL-8 generation, for which TNF-α induced maximal response, the greatest effect observed on MCP-1 generation was with IL-1α (Figure 4). In addition, cotreatment of HLE with increasing concentrations of IL-1α (0.01 to 10 U/ml) and IFN-γ (50 U/ml) demonstrated a synergistic effect of IFN-γ on MCP-1 generation with 0.01 and 0.1 U/ml of IL-1α. This synergism was not observed with the higher concentrations of IL-1α (Figure 4). LPS was much less effective than IL-1α, TNF-α, or IFN-γ at inducing MCP-1 generation by HLE (Figure 4).

Interestingly, in combination with either TNF-α or LPS, IFN-γ synergistically increased MCP-1 release compared with either TNF-α or LPS alone (Table 2). However, no synergistic effect was observed for IL-8 generation after treatment with either TNF-α or LPS in combination with IFN-γ (Table 2). Thus, IFN-γ alone is unable to induce IL-8 generation and only has a synergistic effect in combination with IL-1. These results clearly indicate differential, independent cytokine upregulation of IL-8 and MCP-1 generation in microvascular endothelial cells with the effective order of maximal response for IL-8 generation being TNF ≫ IL-1α > LPS and no effect with IFN-γ. For MCP-1, the order of potency was IL-1α ≫ TNF-α > IFN-γ > LPS.

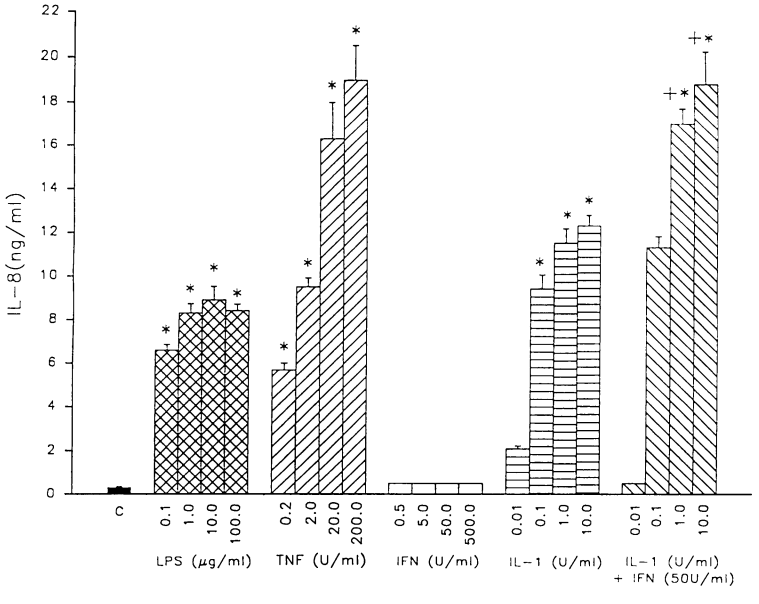

Figure 3. *IL-8 generation by cytokine and LPS-stimulated HLE cells. Antigenic IL-8 levels were determined by ELISA after 24 hours of stimulation with appropriate doses of LPS, TNF-α, IFN-γ, IL-1α, IL-1α and IFN-γ, or medium control (c). Results are the mean ± SEM (n = 8), all experiments were performed in quadruplet. *Values significantly different from medium control (c), P < 0.05. +Values are significantly different (P < 0.05) compared with corresponding IL-1-treated cells. One-way analysis of variance, Bonferroni modified t-test.*

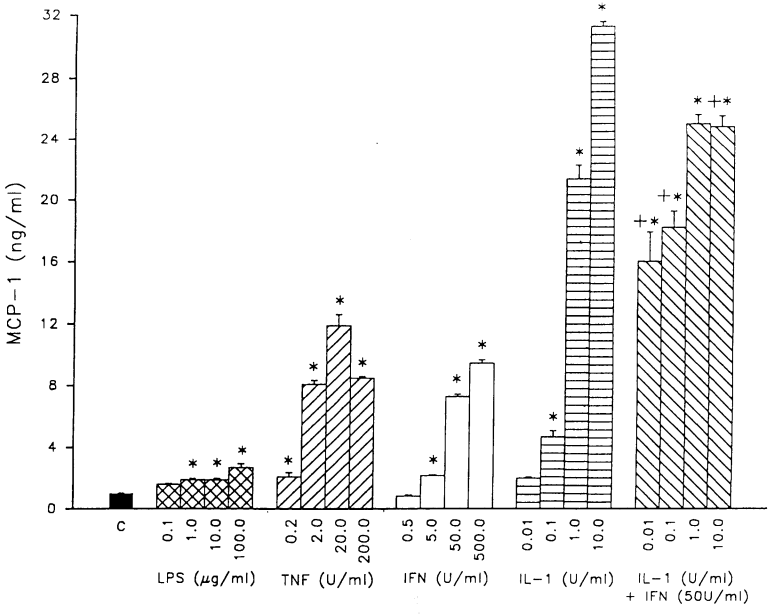

Figure 4. *Dose-dependent induction of MCP-1 generation by cytokine and LPS-activated HLE cells. The HLE cells were challenged with the appropriate doses of LPS, TNF-α, IFN-γ, IL-1α, IL-1α and IFN-γ, or medium control (c) for 24 hours and supernatants were quantitated for antigenic MCP-1 levels by ELISA. Results are the mean ± SEM (n = 8), all experiments were performed in quadruplet. *Values significantly different from medium control (c) P < 0.05. †Values are significantly different (P < 0.05) compared with corresponding IL-1-treated cells. One-way analysis of variance, followed by Bonferroni modified t-test.*

Table 2. *The Effect of IFN-γ on TNF-α and LPS-Stimulated IL-8 and MCP-1 Generation by HLE Cells*

Treatment	IL-8 (ng/ml) Mean ± SEM	MCP-1 (ng/ml) Mean ± SEM
TNF-α (20 U/ml)	22.0 ± 1.2	14.8 ± 0.4
	NS	*
TNF + IFN-γ (50 U/ml)	20.0 ± 2.2	35.0 ± 0.7
IFN-γ (50 U/ml)	<0.3	7.3 ± 0.1
LPS (10 μg/ml)	7.9 ± 0.7	2.9 ± 0.3
	NS	*
LPS + IFN-γ (50 U/ml)	3.6 ± 0.2	16.4 ± 0.8
IFN-γ (50 U/ml)	<0.3	7.3 ± 0.1

NS, not significant.
* Values significantly different $P < 0.05$. One-way analysis of variance by post hoc Bonferroni *t*-test. Values are mean ± SEM of four separate determinations.

Discussion

The results of this study demonstrate that cultured human lung microvascular endothelial cells on stimulation with the appropriate cytokine can express the genes for and secrete the chemokines IL-8 and MCP-1. This is the first demonstration that microvascular endothelium, similar to their large vessel counterparts, can produce the chemokines that may be critical in the recruitment of leukocytes from the blood compartment.

IL-8 is produced by most cell types in the lung, including alveolar macrophages, fibroblasts, and alveolar epithelial cells.[33–35] In cells such as the fibroblast, epithelia and microvascular endothelia, the increased synthesis of IL-8 must be preceded by an initial host response. Kunkel et al[33] suggested that in the context of the lung, the alveolar macrophage is likely to play a major role by generating cytokines such as IL-1 and TNF-α. IFN-γ, primarily considered a product of T lymphocytes, may also be produced by pulmonary alveolar macrophages.[36] Local generation of these inflammatory/immune cytokines can thus lead to increased production of IL-8 and other chemokines by lung fibroblasts, type II epithelial cells, and endothelial cells. This cascade of cytokine network is likely to be a major contributor to the recruitment of specific inflammatory cells. Recent studies of Antoniades et al[37] provide *in vivo* evidence for the expression of MCP-1 mRNA and protein product in pulmonary epithelial, macrophages, vascular endothelial, and smooth muscle cells of the small artery in human idiopathic pulmonary fibrosis (IPF) disease. In the control non-IPF group only the epithelial cells remained negative for MCP-1 mRNA expression. This study demonstrates that the endothelial cells of the small artery in non-IPF and IPF specimens is a good source of MCP-1 mRNA and its protein product; the authors suggest that this may represent a mechanism for the physiological recruitment of circulating monocytes in acute injury.[37] In our study unstimulated HLE cells expressed undetectable levels of IL-8 mRNA, however, a low level of constitutive expression of MCP-1 mRNA transcripts was observed.

Generally, IL-8 is not considered an anti-inflammatory factor. However, Hechtman et al[13] recently provided evidence that extravascular IL-8 elicits PMN emigration, whereas intravascular IL-8 may impair leukocyte adhesion and thus play a protective role against PMN-mediated injury. Although it is

unclear whether IL-8 secretion from endothelial cells exhibits selective polarity, it is conceivable that IL-8 generation by the pulmonary microvascular endothelium could shift the large marginated pool of these leukocytes from the lung to the circulating pool. Thus, IL-8 produced by pulmonary microvascular endothelial cells has the potential to be either proinflammatory or anti-inflammatory in its effects.

Regulation of IL-8 gene expression in HLE appears to be similar to that reported in HUVEC, eg, Rollins et al[19] observed that IFN-γ induced MCP-1 mRNA in HUVEC but did not induce IL-8 neutrophil-activating peptide-1 mRNA. Comparisons of the dose dependence of different cytokines on endothelial IL-8 generation in a single study have not to the best of our knowledge been reported to date and therefore comparisons in the responses to different cytokines with HUVEC or other endothelia cannot be made at this juncture. The observation that IFN-γ can act synergistically with IL-1 suggests that IFN-γ augments some common step in the activation of IL-8 and MCP-1 gene expression. IFN-γ is known to increase endothelial intercellular adhesion molecule-1 and major histocompatibility complex class II expression in response to TNF-α.[21] Johnson and Pober[38] have suggested that this synergism results from an increase in the rate of specific transcriptional factors regulating these genes.

The ability of IFN-γ to selectively stimulate MCP-1 production but not IL-8 has also been observed in human renal cortical epithelial cells.[38] Likewise, IFN-γ fails to induce expression of the IL-8 gene in peripheral blood mononuclear cells even though it induces high level expression of the related gene γIP-10.[29] Thus, it would appear that differential expression of chemokines in these cells may be selectively controlled by IFN-γ. By selectively secreting either a monocyte (MCP-1) or a neutrophil (IL-8) chemotactic factor, it may be possible for HLE to regulate the recruitment of different types of peripheral blood inflammatory cells. The mechanisms controlling the differential regulation of these chemokines are not yet defined, however, a number of transcription regulatory elements for these genes have been sequenced. The 5′-flanking region of the IL-8 gene contains several potential transcription regulatory elements including nuclear factor$_\kappa$B, activator protein-1, activator protein-2, nuclear factor IL-6 binding site, hepatocyte nuclear factor-1, glucocorticoid receptor, and IFN regulatory factor-1.[40] Further analysis revealed that IL-8 gene expression induced by IL-1, TNF, or phorbol myristate acetate required the cooperation of two distinct *cis*-elements, NF-κB and C/EBP-like factor, suggesting that all three stimuli modulate the identical combination of nuclear factors possibly via phosphorylation of a common serine protein kinase.[41] Interestingly, the upstream region of the MCP-1 gene in humans differs from the corresponding region of the IL-8 gene, however, the potential transcriptional regulatory elements for both chemokines are similar.[40,42,43] In our study we observed a disparate response of chemokine production by IFN-γ-stimulated HLE, suggesting that there must be as yet undefined elements of specificity. Alternatively, there may be differences in signaling proteins or associated additional binding that dictate cytokine-specific effects in different cell types. Preferential regulation of certain genes by IFN-γ has been described by Wright et al.[44] In this study they demonstrate selective transcriptional activation of the cytokine gene, mig, by IFN-γ but not IFN-α.

In conclusion, our study demonstrates the secretion of the archetype chemokines IL-8 and MCP-1 by cytokine-activated pulmonary microvascular endothelial cells and provides the basis for the accumulation of both neutrophils and monocytes at sites of vascular injury and inflammation. Despite the similarities between IL-8 and MCP-1, there are many differences in the controls governing their inducible expression and secretion in HLE cells: 1) the induction of MCP-1 by IFN-γ *versus* the lack of induction of IL-8; 2) IL-1α was the most potent inducer of MCP-1 generation, whereas TNF-α induced maximal IL-8 secretion; 3) time course studies show differences in the secretion profile for IL-8 and MCP-1 after IL-1 activation of HLE cells; and 4) IL-1α, TNF-α, or LPS in combination with IFN-γ were synergistic for MCP-1 secretion; however, in the case of IL-8 a synergistic response was only observed with IL-1 and IFN-γ. Overall, these difference in chemokine secretion by pulmonary microvascular endothelial cells may explain the specific appearance of various leukocytes at sites of inflammation and injury.

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